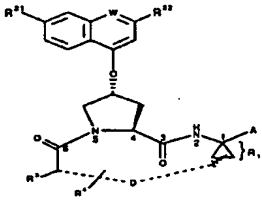




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| <b>(51) International Patent Classification <sup>7</sup> :</b><br><b>C07K 5/08, 5/078, A61K 38/05, 38/06, A61P 31/14</b>  | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 00/59929</b><br><b>(43) International Publication Date:</b> 12 October 2000 (12.10.00)  |
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| <b>(54) Title:</b> MACROCYCLIC PEPTIDES ACTIVE AGAINST THE HEPATITIS C VIRUS  |           |   |
| <div style="text-align: center;">  <p>(1)</p> </div>   |           |   |
| <b>(57) Abstract</b><br><p>The present invention covers macrocyclic compounds of formula (1) active in-vitro and in cellular assays against the NS3 protease of the hepatitis C virus wherein W is CH or N; R<sup>21</sup> is H, halo, C<sub>1-6</sub> alkyl, cycloalkyl, haloalkyl, C<sub>1-6</sub> alkoxy, cycloalkoxy, hydroxy, or N(R<sup>23</sup>)<sub>2</sub>, wherein each R<sup>23</sup> is independently H, C<sub>1-6</sub> alkyl or cycloalkoxy; and R<sup>22</sup> is H, halo, C<sub>1-6</sub> alkyl, C<sub>3-6</sub> cycloalkyl, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> thioalkyl, C<sub>1-6</sub> alkoxy, C<sub>3-6</sub> cycloalkoxy, C<sub>2-7</sub> alkoxyalkyl, C<sub>3-6</sub> cycloalkyl, C<sub>6</sub> or 10 aryl or Het, wherein Het is a five-, six-, or seven-membered saturated or unsaturated heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur; said cycloalkyl, aryl or Het being substituted with R<sup>24</sup>, wherein R<sup>24</sup> is H, C<sub>1-6</sub> alkyl, C<sub>3-6</sub> cycloalkyl, C<sub>1-6</sub> alkoxy, C<sub>3-6</sub> cycloalkoxy, NO<sub>2</sub>, N(R<sup>25</sup>)<sub>2</sub>, NH-C(O)-R<sup>25</sup>; or NH-C(O)-NH-R<sup>25</sup>, wherein each R<sup>25</sup> is independently: H, C<sub>1-6</sub> alkyl or C<sub>3-6</sub> cycloalkyl; or R<sup>24</sup> is NH-C(O)-OR<sup>26</sup> wherein R<sup>26</sup> is C<sub>1-6</sub> alkyl or C<sub>3-6</sub> cycloalkyl; R<sup>3</sup> is hydroxy, NH<sub>2</sub>, or a group of formula -NH-R<sup>31</sup>, wherein R<sup>31</sup> is C<sub>6</sub> or 10 aryl, heteroaryl, -C(O)-R<sup>32</sup>, -C(O)-OR<sup>32</sup> or -C(O)-NHR<sup>32</sup>, wherein R<sup>32</sup> is: C<sub>1-6</sub> alkyl or C<sub>3-6</sub> cycloalkyl; D is a 5 to 10-atom saturated or unsaturated alkylene chain optionally containing one to three heteroatoms independently selected from: O, S, or N-R<sup>41</sup>, wherein R<sup>41</sup> is H, C<sub>1-6</sub> alkyl, cycloalkyl or -C(O)-R<sup>42</sup>, wherein R<sup>42</sup> is C<sub>1-6</sub> alkyl, cycloalkyl or C<sub>6</sub> or 10 aryl; R<sup>4</sup> is H or from one to three substituents at any carbon atom of said chain D, said substituent independently selected from the group consisting of: C<sub>1-6</sub> alkyl, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> alkoxy, hydroxy, halo, amino, oxo, thio or C<sub>1-6</sub> thioalkyl, and A is an amide of formula -C(O)-NH-R<sup>5</sup>, wherein R<sup>5</sup> is selected from the group consisting of: C<sub>1-8</sub> alkyl, C<sub>3-6</sub> cycloalkyl, C<sub>6</sub> or 10 aryl or C<sub>7-16</sub> aralkyl; or A is a carboxylic acid or a pharmaceutically acceptable salt or ester thereof.</p> |           |   |

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## MACROCYCLIC PEPTIDES ACTIVE AGAINST THE HEPATITIS C VIRUS

### FIELD OF THE INVENTION

The present invention relates to compounds, compositions, the preparation of such  
5 compounds and methods for the treatment of hepatitis C virus (HCV) infection. In  
particular, the present invention provides novel peptide analogues, pharmaceutical  
compositions containing such analogues and methods for using these analogues in  
the treatment of HCV infection.

### BACKGROUND OF THE INVENTION

10 Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and  
community-acquired non-A non-B hepatitis worldwide. It is estimated that over 170  
million people worldwide are infected by the virus. A high percentage of carriers  
become chronically infected and many progress to chronic liver disease, so-called  
chronic hepatitis C. This group is in turn at high risk for serious liver disease such  
15 as liver cirrhosis, hepatocellular carcinoma and terminal liver disease leading to  
death.

The mechanism by which HCV establishes viral persistence and causes a high rate of  
chronic liver disease has not been thoroughly elucidated. It is not known how HCV  
interacts with and evades the host immune system. In addition, the roles of cellular  
20 and humoral immune responses in protection against HCV infection and disease  
have yet to be established. Immunoglobulins have been reported for prophylaxis of  
transfusion-associated viral hepatitis, however, the Center for Disease Control does  
not presently recommend immunoglobulins treatment for this purpose. The lack of  
an effective protective immune response is hampering the development of a vaccine  
25 or adequate post-exposure prophylaxis measures, so in the near-term, hopes are  
firmly pinned on antiviral interventions.

Various clinical studies have been conducted with the goal of identifying  
pharmaceutical agents capable of effectively treating HCV infection in patients  
afflicted with chronic hepatitis C. These studies have involved the use of interferon-  
30 alpha, alone and in combination with other antiviral agents. Such studies have  
shown that a substantial number of the participants do not respond to these  
therapies, and of those that do respond favorably, a large proportion were found to

relapse after termination of treatment.

Until a few years ago, interferon (IFN) was the only available therapy of proven benefit approved in the clinic for patients with chronic hepatitis C. However the sustained response rate is low, and interferon treatment also induces severe side-effects (i.e. retinopathy, thyroiditis, acute pancreatitis, depression) that diminish the quality of life of treated patients. Interferon in combination with ribavirin was originally approved for patients non-responsive to IFN alone. It has now been approved for naïve patients and presently constitutes the gold standard in HCV therapy. However, the side effects caused by IFN are not alleviated with this combination therapy.

Therefore, a need exists for the development of effective antiviral agents for treatment of HCV infection that overcomes the limitations of existing pharmaceutical therapies.

HCV is an enveloped positive strand RNA virus in the Flaviviridae family. The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non-structural (NS) proteins. In the case of HCV, the generation of mature nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one, as yet poorly characterized, cleaves at the NS2-NS3 junction; the second one is a serine protease contained within the N-terminal region of NS3 (henceforth referred to as NS3 protease) and mediates all the subsequent cleavages downstream of NS3, both in *cis*, at the NS3-NS4A cleavage site, and in *trans*, for the remaining NS4A-NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protein with NS4A seems necessary to the processing events, enhancing the proteolytic efficiency at all of the sites. The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. NS5B is a RNA-dependent RNA polymerase that is involved in the replication of HCV.

Patent application WO 97/06804 describes the (-) enantiomer of the nucleoside

analogue cytosine-1,3-oxathiolane (also known as 3TC) as active against HCV. This compound, although reported as safe in previous clinical trials against HIV and HBV, has yet to be clinically proven active against HCV and its mechanism of action against the virus has yet to be reported.

- 5 A general strategy for the development of antiviral agents is to inactivate virally encoded enzymes that are essential for the replication of the virus
- In this vein, intense efforts to discover compounds which inhibit the NS3 protease or RNA helicase of HCV have led to the following disclosures:
- US patent 5,633,388 describes heterocyclic-substituted carboxamides and analogues
- 10 as being active against HCV. These compounds are directed against the helicase activity of the NS3 protein of the virus but clinical tests have not yet been reported. A phenanthrenequinone has been reported by Chu et al., (Tet. Lett., (1996), 7229-7232) to have activity against the HCV NS3 protease *in vitro*. No further development on this compound has been reported.
- 15 A paper presented at the Ninth International Conference on Antiviral Research, Urabandai, Fukuyshima, Japan (1996) (Antiviral Research, (1996), 30, 1, A23 (abstract 19)) reports thiazolidine derivatives to be inhibitory to the HCV protease. Several studies have reported compounds inhibitory to other serine proteases, such as human leukocyte elastase. One family of these compounds is reported in WO
- 20 95/33764 (Hoechst Marion Roussel, 1995). The peptides disclosed in that application are morpholinylcarbonyl-benzoyl-peptide analogues that are structurally different from the peptides of the present invention.
- WO 98/17679 from Vertex Pharmaceuticals Inc. discloses inhibitors of serine protease, particularly, Hepatitis C virus NS3 protease
- 25 Hoffman LaRoche (WO 98/22496; US 5,866,684 & US 6,018,020) has also reported hexapeptides that are proteinase inhibitors useful as antiviral agents for the treatment of HCV infection.
- Steinkühler *et al.* and Ingallinella *et al.* have published on NS4A-4B product inhibition (Biochemistry (1998), 37, 8899-8905 and 8906-8914).
- 30 WO 97/43310 by Schering Corporation discloses 20 and 21 amino acid peptide sequences active against the HCV NS3 protease.
- WO 98/46597 by Emory University discloses peptides and peptidomimetics active

*in vitro* against serine proteases.

WO 98/46630 by Peptide Therapeutics Limited discloses depsipeptide substrate inhibiting the HCV NS3 protease.

Finally, US 5,869,253 discloses enzymatic RNA molecules that inhibit the HCV NS3 protease.

None of the prior patent applications described above disclose suggest cyclic peptides active and selective against the Hepatitis C virus NS3 protease.

WO 99/07733, WO 99/07734, WO 00/09543 and WO00/09558 disclose hexa to tetra-peptides and tripeptide analogs that inhibit the NS3 protease. However, these disclosures do not suggest or lead to the design of macrocyclic analogs of the present invention.

WO 99/38888 published August 5, 1999 by the Institute de Recherche di Biologia Moleculare (IRBM) discloses small peptides inhibitors of the HCV NS3 protease. Nothing in this disclosure suggest or indicates the cyclic nature of the peptides of the present invention. In addition, this PCT application was published after the priority date of the present application.

WO 99/64442 by IRBM, also published after the priority date of this application, discloses oligopeptides with ketoacids at P1.

WO 99/50230 from Vertex Pharmaceuticals (published on October 7, 1999) was also published after the priority date of the present application. Even then, the publication does not remotely suggest any cyclic peptides of the present invention.

One advantage of the present invention is that it provides macrocyclic peptides that are inhibitory to the NS3 protease of the hepatitis C virus.

A further advantage of one aspect of the present invention resides in the fact that these peptides specifically inhibit the NS3 protease and do not show significant inhibitory activity against other serine proteases such as human leukocyte elastase (HLE), porcine pancreatic elastase (PPE), or bovine pancreatic chymotrypsin, or cysteine proteases such as human liver cathepsin B (Cat B).

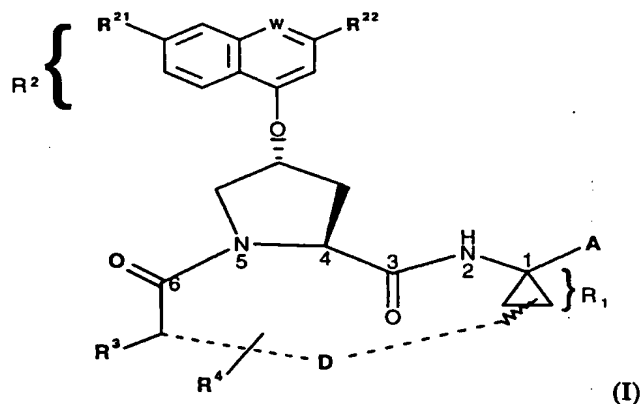
A further advantage of the present invention is that it provides small peptides of low molecular weight that are capable of penetrating cell membranes and inhibit the NS3 protease activity in cell culture.

Still, a further advantage of the compounds of the present invention resides in the fact that they are active in both major genotypes found in clinical isolates (1a & 1b), strongly suggesting that these compound will be active against all presently known genotypes of HCV.

5

# SUMMARY OF THE INVENTION

Included in the scope of the invention are compounds of formula (I):



wherein W is CH or N,

10  $R^{21}$  is H, halo,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkoxy,  $C_{3-6}$  cycloalkoxy, hydroxy, or  $N(R^{23})_2$ , wherein each  $R^{23}$  is independently H,  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl; and

$R^{22}$  is H, halo,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  thioalkyl,  $C_{1-6}$  alkoxy,  $C_{3-6}$  cycloalkoxy,  $C_{2-7}$  alkoxyalkyl,  $C_{3-6}$  cycloalkyl,  $C_{6 \text{ or } 10}$  aryl or Het, wherein Het is a  
 15 five-, six-, or seven-membered, saturated or unsaturated heterocycle, containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur; said cycloalkyl, aryl or Het being substituted with  $R^{24}$ ,

wherein  $R^{24}$  is H, halo,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{1-6}$  alkoxy,  $C_{3-6}$  cycloalkoxy,  $NO_2$ ,  $N(R^{25})_2$ ,  $NH-C(O)-R^{25}$ , or  $NH-C(O)-NH-R^{25}$ ,

20 wherein each  $R^{25}$  is independently: H,  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl; or  $R^{24}$  is  $NH-C(O)-OR^{26}$  wherein  $R^{26}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;

## 6

$R^3$  is hydroxy,  $NH_2$ , or a group of formula  $-NH-R^{31}$ , wherein  $R^{31}$  is  $C_{6 \text{ or } 10}$  aryl, heteroaryl,  $-C(O)-R^{32}$ ,  $-C(O)-OR^{32}$ , or  $-C(O)-NHR^{32}$ , wherein  $R^{32}$  is:  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;

D is a 5 to 10-atom saturated or unsaturated alkylene chain optionally containing one to three heteroatoms independently selected from: O, S, or  $N-R^{41}$ , wherein  $R^{41}$  is H,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl, or  $-C(O)-R^{42}$ , wherein  $R^{42}$  is  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl or  $C_{6 \text{ or } 10}$  aryl;

$R^4$  is H or from one to three substituents at any carbon atom of said chain D, said substituent independently selected from the group consisting of:  $C_{1-6}$  alkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkoxy, hydroxy, halo, amino, oxo, thio, or  $C_{1-6}$  thioalkyl and

A is an amide of formula  $-C(O)-NH-R^5$ , wherein  $R^5$  is selected from the group consisting of:  $C_{1-8}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{6 \text{ or } 10}$  aryl or  $C_{7-16}$  aralkyl; or A is a carboxylic acid or a pharmaceutically acceptable salt or ester thereof.

15

Included within the scope of this invention is a pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I, or a therapeutically acceptable salt or ester thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

20 An important aspect of the invention involves a method of treating a hepatitis C viral infection in a mammal by administering to the mammal an anti-hepatitis C virally effective amount of the compound of formula I, or a therapeutically acceptable salt or ester thereof or a composition as described above.

Another important aspect involves a method of inhibiting the replication of hepatitis C virus by exposing the virus to a hepatitis C NS3 protease-inhibiting amount of the compound of formula I, or a therapeutically acceptable salt or ester thereof or a composition as described above.

25

Still another aspect involves a method of treating a hepatitis C viral infection in a mammal by administering thereto an anti-hepatitis C virally effective amount of a



combination of the compound of formula I, or a therapeutically acceptable salt or ester thereof. According to one embodiment, the pharmaceutical compositions of this invention comprise an additional immunomodulatory agent. Examples of additional immunomodulatory agents include but are not limited to,  $\alpha$ -,  $\beta$ -, and  $\delta$ -interferons.

According to an alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an antiviral agent. Examples of antiviral agents include, ribavirin and amantadine.

According to another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise other inhibitors of HCV protease.

According to yet another alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase, metalloprotease or IRES.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

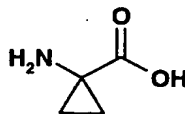
### Definitions

As used herein, the following definitions apply unless otherwise noted:

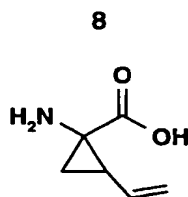
With reference to the instances where (*R*) or (*S*) is used to designate the absolute configuration of a substituent, e.g.  $R^4$  of the compound of formula I, the designation is done in the context of the whole compound and not in the context of the substituent alone.

The designation "P1, P2, and P" as used herein refer to the position of the amino acid residues starting from the C-terminus end of the peptide analogs and extending towards the N-terminus (i.e. P1 refers to position 1 from the C-terminus, P2: second position from the C-terminus, etc.) (see Berger A. & Schechter I., Transactions of the Royal Society London series B257, 249-264 (1970)).

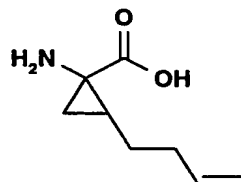
As used herein the term "1-aminocyclopropyl-carboxylic acid" (ACCA) refers to a compound of formula:



As used herein the term "vinyl-ACCA" refers to a compound of formula:



As used herein the term "homo-allyl-ACCA" refers to a compound of formula:



The term "halo" as used herein means a halogen substituent selected from bromo,  
5 chloro, fluoro or iodo.

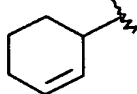
The term "C<sub>1-6</sub> haloalkyl" as used herein means as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl substituents containing from 1 to six carbon atoms having one or more hydrogen substituted for a halogen selected from bromo, chloro, fluoro or iodo.

10 The term "C<sub>1-6</sub> thioalkyl" as used herein means as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl substituents containing a thiol group such a thiopropyl.

The term "C<sub>1-6</sub> alkyl" or "(lower)alkyl" as used herein, either alone or in combination with another substituent, means acyclic, straight or branched chain alkyl  
15 substituents containing from 1 to six carbon atoms and includes, for example, methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl.

The term "C<sub>3-6</sub> cycloalkyl" as used herein, either alone or in combination with another substituent, means a cycloalkyl substituent containing from three to six  
20 carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

The term "unsaturated cycloalkyl" includes, for example, the substituent cyclohexenyl:



The term "saturated or unsaturated alkylene" as used herein means a divalent alkyl substituent derived by the removal of one hydrogen atom from each end of a saturated or unsaturated straight or branched chain aliphatic hydrocarbon and includes, for example,

- 5 -CH<sub>2</sub>CH<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-, -CH<sub>2</sub>CH<sub>2</sub>CH=CHCH<sub>2</sub>CH<sub>2</sub>- or -CH<sub>2</sub>C≡CCH<sub>2</sub>CH<sub>2</sub>-. This alkyl chain may optionally contain a heteroatom such as oxygen (for example: CH<sub>3</sub>-CH<sub>2</sub>-O-CH<sub>2</sub>-).

- The term "C<sub>1-6</sub> alkoxy" as used herein, either alone or in combination with another substituent, means the substituent -O-C<sub>1-6</sub> alkyl wherein alkyl is as defined above containing up to six carbon atoms. Alkoxy includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter substituent is known commonly as *tert*-butoxy.
- 10

- The term "C<sub>3-6</sub> cycloalkyl" as used herein, either alone or in combination with another substituent, means the substituent -O-C<sub>3-6</sub> cycloalkyl containing from three to 6 carbon atoms.
- 15

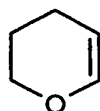
The term "C<sub>1-6</sub> alkoxyalkyl" as used herein, means the substituent C<sub>1-6</sub> alkyl-O-C<sub>1-6</sub> alkyl wherein alkyl is as defined above containing up to six carbon atoms. For example, methoxymethyl means -CH<sub>2</sub>-O-CH<sub>3</sub>.

- The term "C<sub>2-7</sub> acyl" as used herein, either alone or in combination with another substituent, means an C<sub>1-6</sub> alkyl group linked through a carbonyl group such as -C(O)-C<sub>1-6</sub> alkyl.
- 20

- The term "C<sub>6</sub> or C<sub>10</sub> aryl" as used herein, either alone or in combination with another substituent, means either an aromatic monocyclic system containing 6 carbon atoms or an aromatic bicyclic system containing 10 carbon atoms. For example, aryl includes a phenyl or a naphthyl - ring system.
- 25

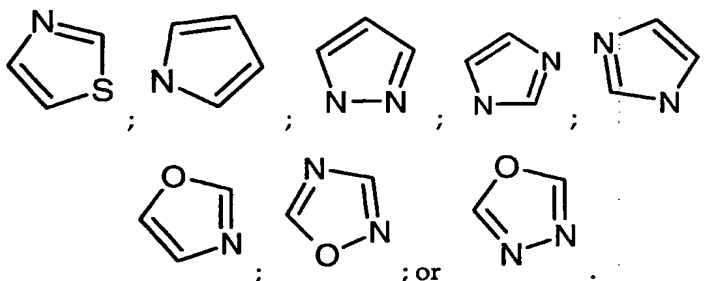
The term "C<sub>7-16</sub> aralkyl" as used herein, either alone or in combination with another substituent, means an aryl as defined above linked through an alkyl group, wherein alkyl is as defined above containing from 1 to 6 carbon atoms. Aralkyl includes for example benzyl, and butylphenyl.

The term "Het" as used herein, either alone or in combination with another substituent, means a monovalent substituent derived by removal of a hydrogen from a five-, six-, or seven-membered saturated or unsaturated (including aromatic) heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur. Examples of suitable heterocycles include: tetrahydrofuran, thiophene, diazepine, isoxazole, piperidine, dioxane, morpholine, pyrimidine or

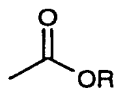


The term "Het" also includes a heterocycle as defined above fused to one or more other cycle be it a heterocycle or any other cycle. One such examples includes thiazolo[4,5-b]-pyridine.

Although generally covered under the term "Het", the term "heteroaryl" as used herein precisely defines an unsaturated heterocycle for which the double bonds form an aromatic system. Suitable example of heteroaromatic system include: quinoline, indole, pyridine,



The term "pharmaceutically acceptable ester" as used herein, either alone or in combination with another substituent, means esters of the compound of formula I in which any of the carboxyl functions of the molecule, but preferably the carboxy terminus, is replaced by an alkoxycarbonyl function:



in which the **R** moiety of the ester is selected from alkyl (e.g. methyl, ethyl, *n*-propyl, *t*-butyl, *n*-butyl); alkoxyalkyl (e.g. methoxymethyl); alkoxyacyl (e.g. acetoxymethyl); aralkyl (e.g. benzyl); aryloxyalkyl (e.g. phenoxyethyl); aryl (e.g. phenyl),

optionally substituted with halogen,  $C_{1-4}$  alkyl or  $C_{1-4}$  alkoxy. Other suitable prodrug esters are found in Design of prodrugs, Bundgaard, H. Ed. Elsevier (1985) incorporated herewith by reference. Such pharmaceutically acceptable esters are usually hydrolyzed *in vivo* when injected in a mammal and transformed into the acid form of the compound of formula I.

With regard to the esters described above, unless otherwise specified, any alkyl moiety present advantageously contains 1 to 16 carbon atoms, particularly 1 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group.

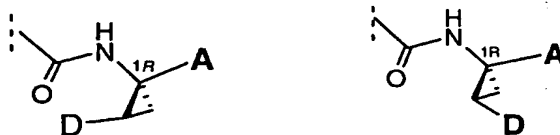
- 10 In particular the esters may be a  $C_{1-16}$  alkyl ester, an unsubstituted benzyl ester or a benzyl ester substituted with at least one halogen,  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, nitro or trifluoromethyl.

The term "pharmaceutically acceptable salt" as used herein includes those derived from pharmaceutically acceptable bases. Examples of suitable bases include choline, ethanolamine and ethylenediamine.  $Na^+$ ,  $K^+$ , and  $Ca^{++}$  salts are also contemplated to be within the scope of the invention (also see Pharmaceutical salts, Birge, S.M. et al., J. Pharm. Sci., (1977), 66, 1-19, incorporated herein by reference).

#### Preferred embodiments

$R^1$ :

- 20 Preferred embodiments of the present invention include compounds of formula I as described above, wherein the  $R^1$  moiety is selected from the 2 different diastereoisomers where the 1-carbon center has the *R* configuration as represented by structures (i) and (ii):



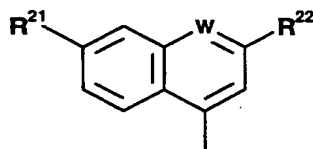
- 25  $D$  syn to the amide (i), or  $D$  syn to A (ii).

More preferably, the linker  $D$  is linked to  $R^1$  in the configuration *syn* to A as represented by structure (ii).

12

 $R^2$ :

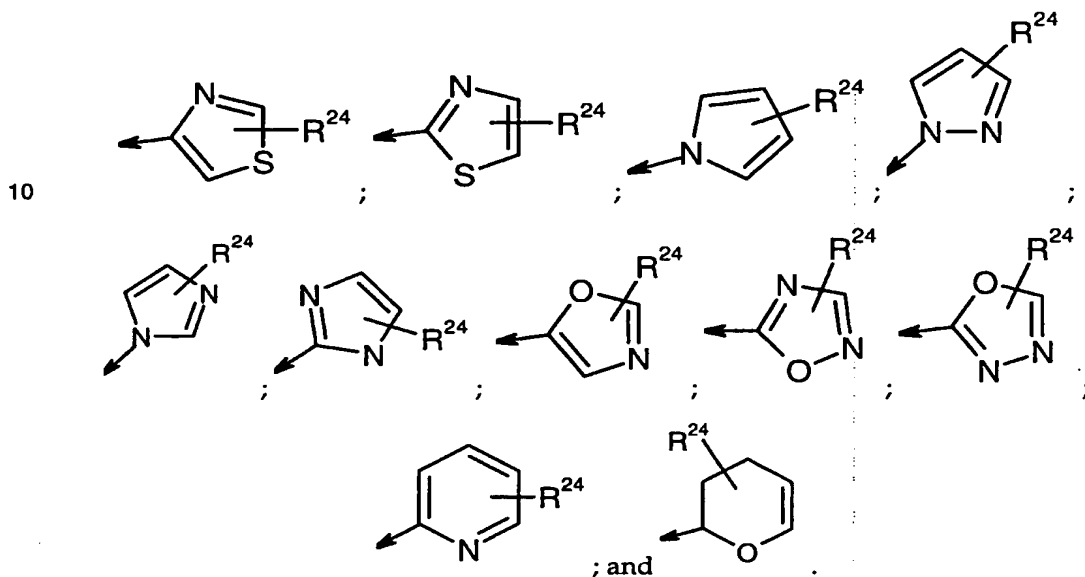
Preferred embodiments of the present invention include compounds of formula I as described above, wherein the  $R^2$  moiety is



wherein W is preferably N.

- 5 Preferably,  $R^{21}$  is H,  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, hydroxy, chloro, or  $N(R^{23})_2$  wherein  $R^{23}$  is preferably H or  $C_{1-6}$  alkyl. More preferably,  $R^{21}$  is H or  $C_{1-6}$  alkoxy. Most preferably,  $R^{21}$  is methoxy.

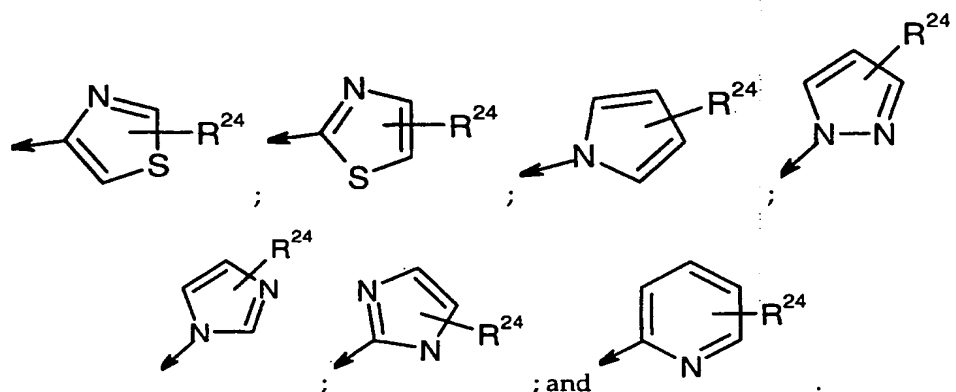
Preferably  $R^{22}$  is H,  $C_{1-6}$  thioalkyl,  $C_{1-6}$  alkoxy, phenyl or Het selected from the group consisting of:



Preferably,  $R^{24}$  is H,  $C_{1-6}$  alkyl,  $NH-R^{25}$ ,  $NH-C(O)-R^{25}$ ; or  $NH-C(O)-NH-R^{25}$  or  $NH-C(O)-OR^{26}$ .

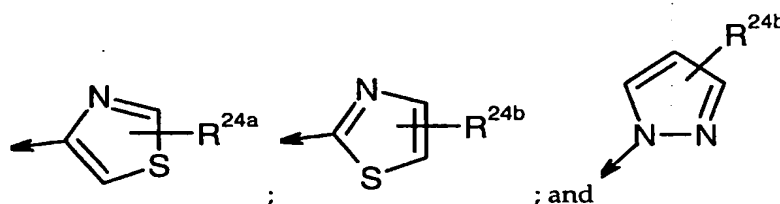
- 15 More preferably  $R^{22}$  is  $C_{1-4}$  alkoxy, phenyl or Het selected from the group consisting of:

13



More preferably,  $R^{24}$  is H,  $C_{1-6}$  alkyl,  $NH-R^{25}$ ,  $NH-C(O)-R^{25}$ , or  $NH-C(O)-OR^{26}$ .

Most preferably  $R^{22}$  is ethoxy, or Het selected from the group consisting of:



5

Most preferably,  $R^{24a}$  is  $NH-R^{25}$ ,  $NH-C(O)-R^{25}$ , or  $NH-C(O)-OR^{26}$ . Most preferably,

$R^{24b}$  is H or  $C_{1-6}$  alkyl.

Preferably, each  $R^{25}$  is independently: H,  $C_{1-6}$  alkyl, or  $C_{3-6}$  cycloalkyl. More

preferably,  $R^{25}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl. More preferably,  $R^{25}$  is  $C_{1-6}$  alkyl.

10 Preferably,  $R^{26}$  is  $C_{1-6}$  alkyl.

$R^3$ :

Preferred embodiments of the present invention include compounds of formula I as described above, wherein the  $R^3$  moiety is preferably an amide of formula  $NH-C(O)-$

15  $R^{32}$ , a urea of formula  $NH-C(O)-NH-R^{32}$ , or a carbamate of formula  $NH-C(O)-OR^{32}$ .

More preferably,  $R^3$  is a carbamate or a urea. Most preferably,  $R^3$  is a carbamate.

Preferably,  $R^{32}$  is  $C_{1-6}$  alkyl, or  $C_{3-6}$  cycloalkyl. More preferably,  $R^{32}$  is  $C_{1-6}$  alkyl, or

$C_{4-6}$  cycloalkyl. Most preferably,  $R^{32}$  is *tert*-butyl, cyclobutyl or cyclopentyl.

D:

Preferred embodiments of the present invention include compounds of formula I, wherein linker D is a 6 to 8 atom saturated or unsaturated alkylene chain. More preferably, linker D is 7 atom chain.

- 5 Preferably, the D chain contains one or two heteroatoms selected from: O, S, NH, N-C<sub>1-6</sub> alkyl or N-C<sub>2-7</sub> acyl. More preferably, the D chain optionally contains one heteroatom selected from: NH, or N-C<sub>2-7</sub> acyl, most preferably N(Ac), and is positioned at atom 10 of the chain. Most preferably, the chain containing a nitrogen atom is saturated.
- 10 Alternatively, D contains one heteroatom selected from: O, or S. Preferably, when D is 7 atom in length, the O or S atom is at position 9 of the chain. Preferably, this chain is substituted with R<sup>4</sup>, wherein R<sup>4</sup> is H or C<sub>1-6</sub> alkyl. More preferably, R<sup>4</sup> is H or methyl. Most preferably, R<sup>4</sup> is H or 8-(S)-Me. Even most preferably, D is saturated. Alternatively, D contains one double bond at position 11,12. Preferably, this double
- 15 bond is *trans*.

Alternatively, D is an all carbon saturated or unsaturated alkylene chain. In this case, D is preferably saturated and is 7 atom in length. More preferably, D is substituted with R<sup>4</sup>, wherein R<sup>4</sup> is H, oxo, thio, hydroxy, thioalkyl, alkoxy or alkyl.

- 20 More preferably, R<sup>4</sup> is H or C<sub>1-6</sub> alkyl. Most preferably, R<sup>4</sup> is H or methyl. Most preferably, R<sup>4</sup> is H or 10-(S)-Me.

- Alternatively, D is an all carbon alkylene chain containing preferably one double bond and is 7 atom in length. More preferably, this double bond is at position 13,14
- 25 of the chain. Most preferably, this double bond is *cis*. Preferably, this D chain is substituted with R<sup>4</sup>, wherein R<sup>4</sup> is H, oxo, hydroxy, alkoxy or alkyl. More preferably, R<sup>4</sup> is H or C<sub>1-6</sub> alkyl. Even more preferably, R<sup>4</sup> is H or methyl. Most preferably, R<sup>4</sup> is H or 10-(S)-Me.

A:

- 30 Preferred embodiments of the present invention include compounds of formula I as



described above, wherein A is a carboxylic acid.

**Specific embodiments:**

Preferred embodiments of the present invention include compounds of formula I as described above, wherein  $R^2$  is a quinoline substituent (i.e. W is N);

- 5  $R^3$  is a group of formula  $-NH-C(O)-NHR^{32}$  or  $-NH-C(O)-OR^{32}$ ,

wherein  $R^{32}$  is:  $C_{1-4}$  alkyl or  $C_{4-6}$  cycloalkyl;

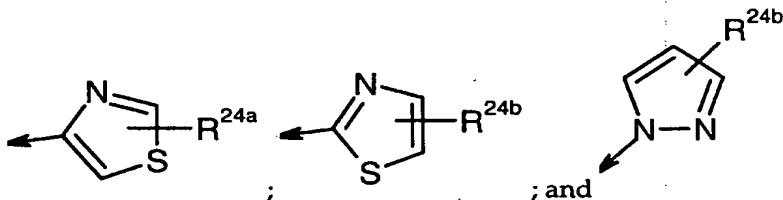
D is a 6 to 8 atom saturated or unsaturated alkylene chain linked to  $R^1$  in configuration *syn* to A, optionally containing one or two heteroatoms independently selected from: O, S or N- $R^{41}$ , wherein  $R^{41}$  is  $C_{2-7}$  acyl;

- 10  $R^4$  is H, or from one to three substituents independently selected from hydroxy or  $C_{1-6}$  alkyl; and

A is a carboxylic acid, or a pharmaceutically acceptable salt or ester thereof.

More preferably are compounds of formula I wherein  $R^1$  is as defined above;  $R^{21}$  is H or methoxy;

- 15  $R^{22}$  is  $C_{1-6}$  alkoxy, or Het selected from the group consisting of:



wherein  $R^{24a}$  is H,  $C_{1-6}$  alkyl,  $NH-R^{25}$ ,  $NH-C(O)-R^{25}$ ,  $NH-C(O)-NH-R^{25}$ ,

wherein  $R^{25}$  is: H,  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;

or  $R^{24a}$  is  $NH-C(O)-OR^{26}$ , wherein  $R^{26}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;

- 20 and  $R^{24b}$  is H or  $C_{1-6}$  alkyl;

$R^3$  is a urea of the formula  $NH-C(O)-NHR^{32}$  or a carbamate of formula  $NH-C(O)-OR^{32}$ , wherein  $R^{32}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;

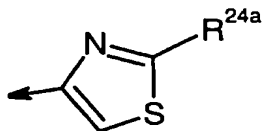
D is a C7-atom saturated or unsaturated alkylene chain optionally containing one double bond at position 11,12 or 13,14;

- 25 said D chain optionally containing one heteroatom independently selected from: O,

S, NH, N(Me), or N(Ac); and

R<sup>4</sup> is H or C<sub>1-6</sub> alkyl.

Most preferably, are compounds of formula I wherein R<sup>21</sup> is methoxy, and R<sup>22</sup> is ethoxy or:



wherein R<sup>24a</sup> is NH-(C<sub>1-4</sub> alkyl), NH-C(O)-(C<sub>1-4</sub> alkyl); or NH-C(O)-O-(C<sub>1-4</sub> alkyl);,

and

D is saturated or contains one *cis* double bond at position 13,14.

Finally, included within the scope of this invention are all compounds of formula I

as presented in Tables 1 to 9.

The pharmaceutical compositions of this invention may be administered orally, parenterally or via an implanted reservoir. Oral administration or administration by injection are preferred. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, and intralesional injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers

which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with  
5 emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Other suitable vehicles or carriers for the above noted formulations and compositions can be found in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", 19<sup>th</sup> ed., Mack Publishing Company, Easton, Penn., 1995.  
10 Dosage levels of between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 75 mg/kg body weight per day of the protease inhibitor compounds described herein are useful in a monotherapy for the prevention and treatment of HCV mediated disease. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 5 times  
15 per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (*w/w*).  
20 Preferably, such preparations contain from about 20% to about 80% active compound.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific  
25 compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small  
30 increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or

deleterious side effects.

When the compositions of this invention comprise a combination of a compound of formula I and one or more additional therapeutic or prophylactic agent, both the compound and the additional agent should be present at dosage levels of between  
5 about 10 to 100%, and more preferably between about 10 and 80% of the dosage normally administered in a monotherapy regimen.

When these compounds or their pharmaceutically acceptable salts are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered *in vivo* to mammals, such as man, to inhibit HCV NS3 protease or to  
10 treat or prevent HCV virus infection. Such treatment may also be achieved using the compounds of this invention in combination with agents which include, but are not limited to: immunomodulatory agents, such as  $\alpha$ -,  $\beta$ -, or  $\gamma$ -interferons; other antiviral agents such as ribavirin, amantadine; other inhibitors of HCV NS3 protease; inhibitors of other targets in the HCV life cycle such as helicase,  
15 polymerase, metalloprotease, or internal ribosome entry site (IRES); or combinations thereof. The additional agents may be combined with the compounds of this invention to create a single dosage form. Alternatively these additional agents may be separately administered to a mammal as part of a multiple dosage form. Accordingly, another embodiment of this invention provides methods of inhibiting  
20 HVC NS3 protease activity in mammals by administering a compound of the formula I, wherein the substituents are as defined above.

In a preferred embodiment, these methods are useful in decreasing HCV NS3 protease activity in a mammal. If the pharmaceutical composition comprises only a compound of this invention as the active component, such methods may  
25 additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV protease inhibitor, or an inhibitor of other targets in the HCV life cycle such as helicase, polymerase, or metalloprotease. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the compositions of this  
30 invention.

In an alternate preferred embodiment, these methods are useful for inhibiting viral replication in a mammal. Such methods are useful in treating or preventing HCV

disease. If the pharmaceutical composition comprises only a compound of this invention as the active component, such methods may additionally comprise the step of administering to said mammal an agent selected from an immunomodulatory agent, an antiviral agent, a HCV protease inhibitor, or an inhibitor of other targets in the HCV life cycle. Such additional agent may be administered to the mammal prior to, concurrently with, or following the administration of the composition according to this invention.

The compounds set forth herein may also be used as laboratory reagents. The Applicant provides for the first time compounds with a low molecular weight, that are highly active and specific against the HCV NS3 protease. Some of the present compounds may be instrumental in providing research tools for designing of viral replication assays, validation of animal assay systems and structural biology studies to further enhance knowledge of the HCV disease mechanisms.

The compounds of this invention may also be used to treat or prevent viral contamination of materials and therefore reduce the risk of viral infection of laboratory or medical personnel or patients who come in contact with such materials (e.g. blood, tissue, surgical instruments and garments, laboratory instruments and garments, and blood collection or transfusion apparatuses and materials).

## 20 Methodology

Several ways of carrying the synthesis of acyclic intermediates of compounds of formula I are disclosed in WO 00/09543 and WO 00/09558.

The compounds of the present invention are synthesized according to the general process illustrated in Schemes I, II and III (wherein PG is an appropriate protecting groups. [In all schemes presented below, D' has the same definition as D but is 2 to 5 atom shorter].

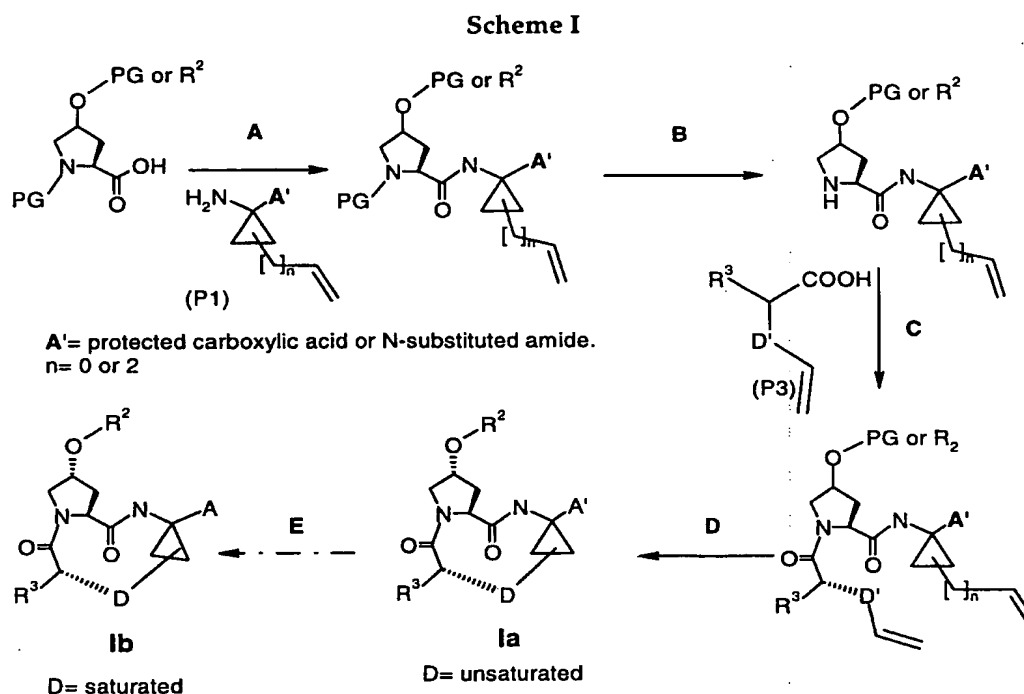
When the invention covers compounds of formula I wherein A is a N-substituted amide, vinyl-ACCA or homo-allyl ACCA ( $R^1$ ) is coupled to an appropriate amine prior to the coupling to P2. Such coupling will be readily recognized by persons skilled in the art. As will be recognized by persons skilled in the art, such amide (A) is not protected but bears any relevant substituent  $R^5$  as defined above.

The ring-closing reaction (macrocyclization) is carried out by either olefin metathesis

(Scheme I) or when the linker contains a nitrogen atom, by reductive amination (Scheme II), or by peptide bond formation Scheme III.

Details of these processes are presented below:

#### 5 A. Macrocyclisation via olefin metathesis

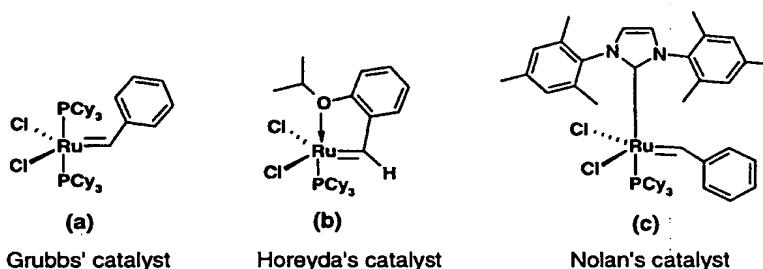


#### 10 Scheme I:

There are several ways in which the coupling sequence can be carried out which can be easily recognized by persons skilled in the art. Starting with 4-(S)-hydroxyproline, the substituent at the 4-hydroxy can be incorporated *via* a Mitsunobu reaction (as described in Mitsunobu *Synthesis* 1981, January, 1-28; Rano *et al. Tet. Lett.* 1994, 36, 3779-3792; Krchnak *et al. Tet. Lett.* 1995, 36, 6193-6196) before or after the macrocyclization. Alternatively the assembly can be done with the required 4-(R)-hydroxy-substituted proline as disclosed in the general processes of WO 00/09543 & WO 00/09558 (see below for specific examples of these fragments).

**Steps A, B, C:** Briefly, the P1, P2, and P3 moieties can be linked by well known peptide coupling techniques and generally disclosed in WO 00/09543 & WO 00/09558.

- Step D:** The formation of the macrocycle can be carried out via an olefin metathesis using a Ru-based catalyst such as the one reported by Miller, S.J.; Blackwell, H.E.; Grubbs, R.H. *J. Am. Chem. Soc.* 1996, 118, 9606-9614 (a); Kingsbury, J.S.; Harrity, J.P.A.; Bonitatebus, P.J.; Hoveyda, A.H. *J. Am. Chem. Soc.* 1999, 121, 791-799 (b) and Huang, J.; Stevens, E.D.; Nolan, S.P.; Petersen, J.L.; *J. Am. Chem. Soc.* 1999, 121, 2674-2678 (c). It will also be recognized that catalysts containing other transition metals such as Mo can be used for this reaction.

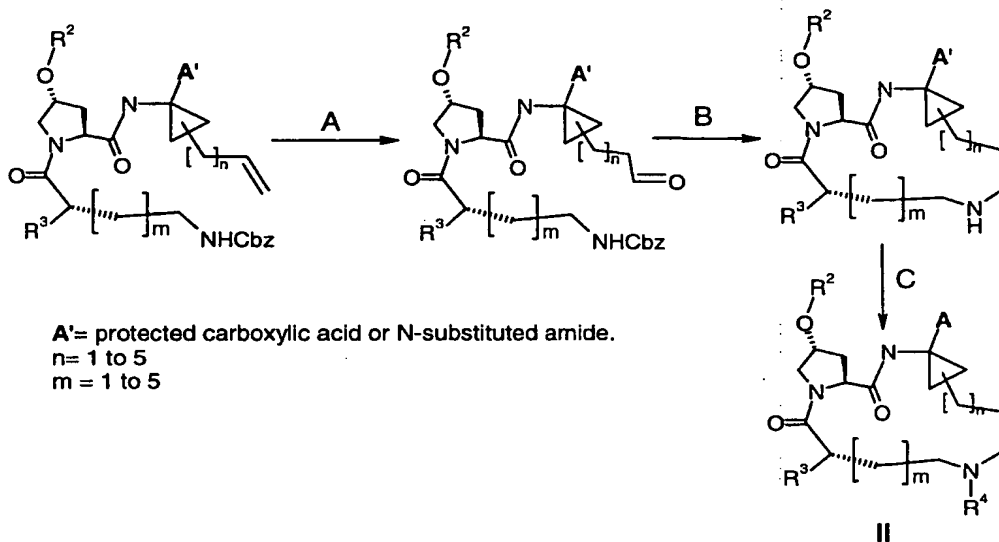


**Step E:** Optionally, the double bond is reduced by standard hydrogenation methods well known in the art. When A' is a protected carboxylic acid, it is also deprotected appropriately.

- 15 B. Macrocyclization via reductive amination (for linkers containing N)**  
When the linker contains a nitrogen atom, macrocyclization is achieved by reductive amination as shown in Scheme II to obtain inhibitors of general structure II.

22

Scheme II



**Step A:** Hydroboration of the double bond following Brown's procedure (H.C.

Brown and B.C. Subba Rao, *J. Am. Chem. Soc.* 1959, 81, 6434-6437) followed by

5 oxidation of the resulting alcohol (for example *via* Dess-Martin periodinate, *J. Am. Chem. Soc.* 1991, 113, 7277-7287) affords the corresponding aldehyde.

**Step B:** Hydrogenation in the presence of acid leads to the removal of the amino protecting group followed by macrocyclization *via* reductive amination.

The P3 unit used in this synthesis is easily obtained from a variety of amino acids, such as lysine, ornithine, glutamine (after a Hofmann reaction: *Ber.* 1881, 14, 2725) and others; these synthetic modifications are methods well known in the art.

**Step C:** Optionally, the secondary amine in the linker D (formed after step D) is alkylated with alkyl halides or acetylated with alkyl or aryl acid chlorides using methodologies well known in the art to obtain inhibitors of general structure II.

15 When A' is a protected carboxylic acid, it is also deprotected appropriately.

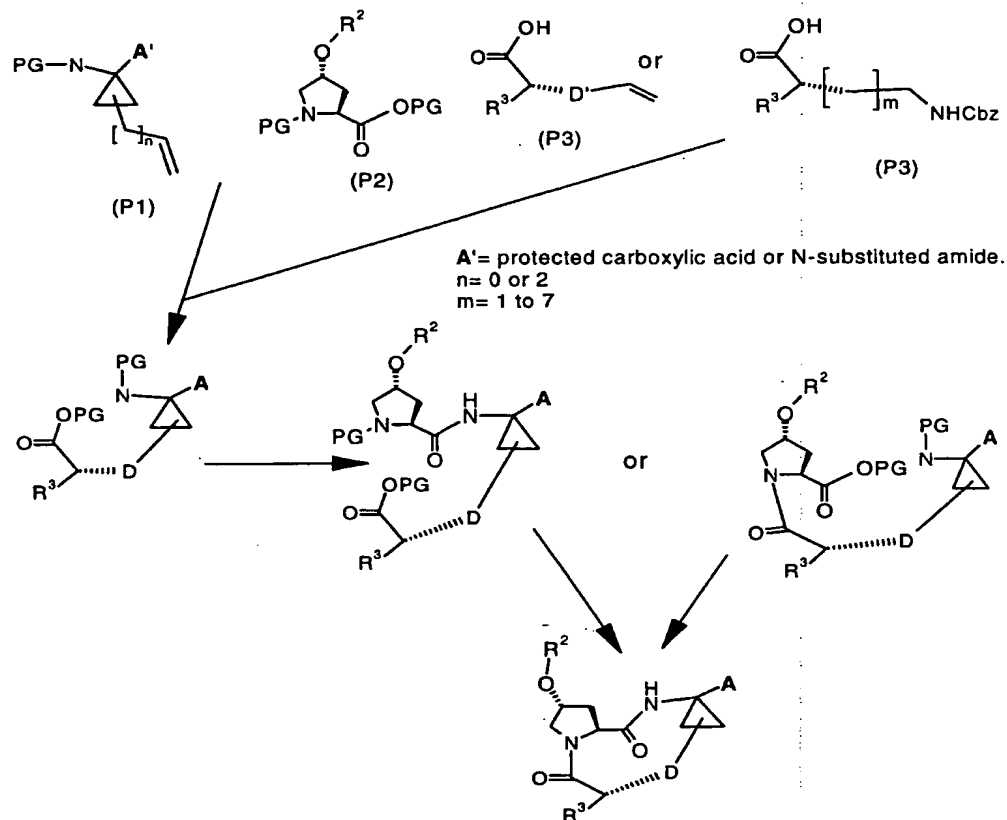
#### C. Macrocyclization via lactam formation

Alternatively, it is understood that these macrocyclic compounds with general structure I and II can be synthesized in other ways. For example P1 and P3 can be first connected to the linker D, then coupled to P2 and the macrocyclization reaction can be a lactam formation in two possible ways as will be recognized by persons skilled in the art and as shown in Scheme III.



## 23

## Scheme III

**Synthesis of P1**

The synthesis of inhibitors with general structure I and II requires the same P1

5 fragments:

- vinyl ACCA, the synthesis and resolution of which is described in WO 00/09543 & WO 00/09558 or
- homoallyl ACCA (Example 1, compound 1f).

**Synthesis of P2**

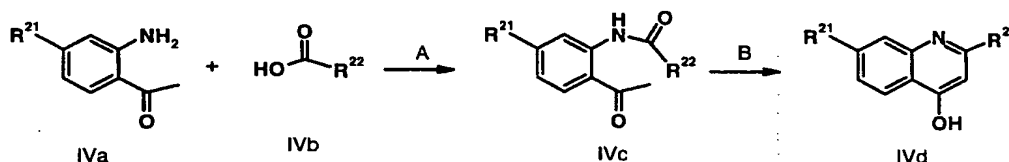
- 10 Some of the P2 fragments used for the synthesis of compounds of formula I are described in WO 00/09543 & WO 00/09558.

Other P2 fragments are synthesized as follows:

- Synthesis of 2-"Het"-4-hydroxy-7-methoxyquinoline derivative**

- 15 (i) Approach from the corresponding "Het" carboxylic acid IVb

Scheme IV



The synthesis is carried out according to a modified procedure in Li *et al.* *J. Med.*

- 5 *Chem.* **1994**, *34*, 3400-3407. Intermediate IVa where  $\text{R}^{21} = \text{OMe}$  (Example 7, compound 7b) is prepared as described by Brown *et al.* *J. Med. Chem.* **1989**, *32*, 807-826.

**Step A:** Intermediate IVa is coupled with heterocyclic carboxylic acids IVb under basic conditions with  $\text{POCl}_3$  to activate the carboxylate group. A variety of

- 10 carboxylic acids with general structure IVb are used for the preparation of inhibitors; these are either commercially available, synthesized as shown in scheme V, VI and VII, or synthesized individually using methods described in the specific examples.

**Step B:** Ring-closure, followed by dehydration is achieved under basic conditions to obtain quinolines of general structure IVd.

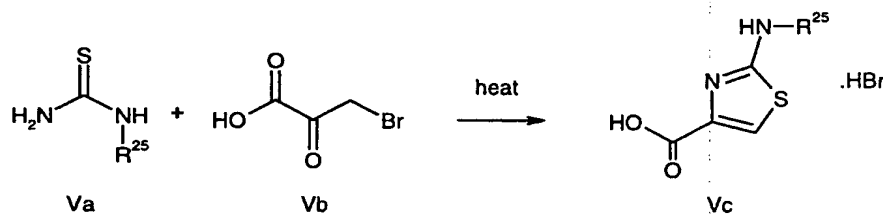
15

(i.a). **Synthesis of "Het"-carboxylic acids of general formula IVb**  
Synthesis of 2-(substituted)-amino-4-carboxy-aminothiazole  
derivatives (Vc)

The modified procedure described by Berdikhina *et al.* *Chem. Heterocycl. Compd.*

- 20 (Engl. Transl.) **1991**, *4*, 427-433 is used.

Scheme V

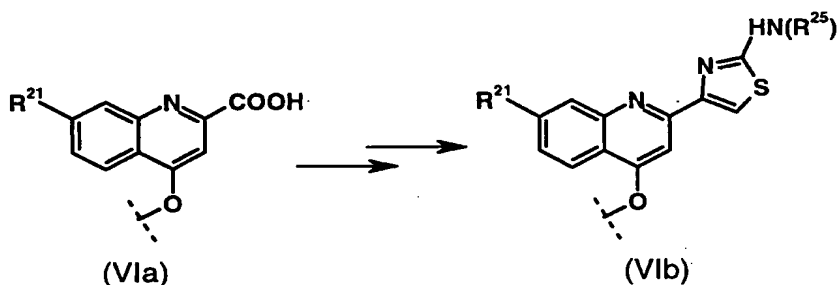


A variety of 2-alkylaminothiazolyl-4-carboxylic acids, compounds of general

- 25 structure Vc, are made using the general synthetic methodology outlined in Scheme

V using thioureas (Va) with different alkyl substituents ( $R^{25}$  = alkyl group) and 3-bromopyruvic acid. This type of condensation reaction is well known in the art. Alternatively, the P2 fragment containing the 2-amino-substituted-thiazole derivatives are synthesized from the corresponding 2-carboxyl derivative as shown in scheme VI according to the procedure of: Unangst, P.C.; Connor, D.T. *J. Heterocyc. Chem.* 29, 5, 1992, 1097-1100.

Scheme VI



Examples of this process are disclosed in WO 00/09543 & WO 00/09558.

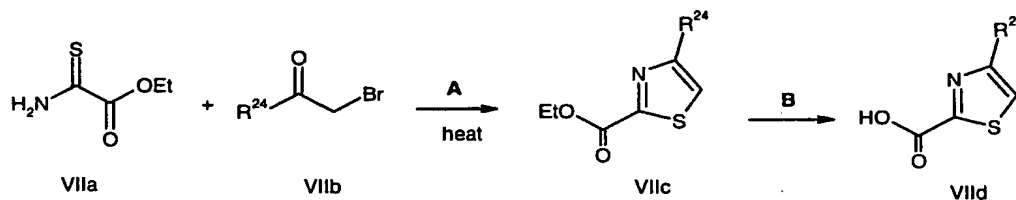
10

Synthesis of 2-carboxy-4-substituted aminothiazole derivatives VIId

A variety of 4-alkylthiazolyl-2-carboxylic acids, compounds of general structure VIId, is made using the general synthetic methodology outlined in Scheme VII.

15

Scheme VII



The procedure described by Janusz *et al.* *J. Med. Chem.* 1998, 41, 3515-3529 is used with modifications as described as follows: Ethyl thiooxamate (VIIa) is reacted with different  $\beta$ -bromoketones of general structure VIIb ( $R^{24}$  = alkyl group) to form thiazolyl carboxylic acids of general structure VIId. This type of condensation reaction is well known in the art.

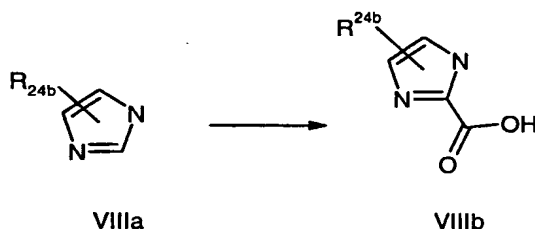
20

Synthesis of 2-carboxy-(substituted)-imidazole derivative  
(VIIIb)

A variety of alkylimidazolyl-2-carboxylic acids, compounds of general structure VIIIb, are made using the general synthetic methodology outlined in Scheme VIII.

5

Scheme VIII

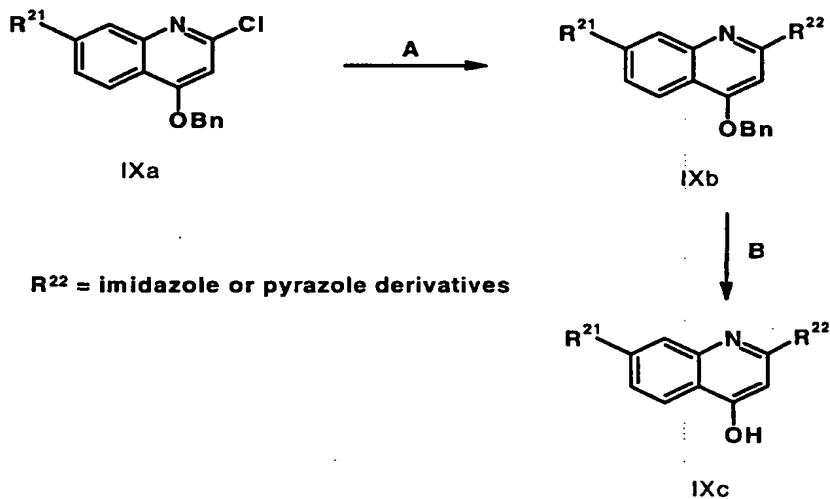


The procedure described by Baird *et al.* *J. Amer. Chem. Soc.* **1996**, *118*, 6141-6146. was used: an alkyl imidazole is deprotonated with a strong base (e.g. *n*BuLi) and then reacted with CO<sub>2</sub> to form the carboxylic acid VIIIb. This type of condensation reaction is well known in the art.

10

**b. Synthesis of 4-hydroxy-7-methoxy-2- (imidazolyl or pyrazolyl)quinolines**  
4-Hydroxy-7-R<sup>21</sup> quinolines having an imidazolyl or pyrazolyl moiety at C2 are generally prepared using the methodology outlined in Scheme IX.

Scheme IX



15

The synthesis of the key intermediate, (wherein R<sup>21</sup> = OMe) 4-benzyloxy-2-chloro-7-methoxyquinoline IXa is described in detail in Example 6 (compound 6e).

**Step A:** At high temperatures, a variety of imidazoles, alkyl substituted imidazoles, pyrazoles or alkyl substituted pyrazoles can be used to displace the 2-chloro moiety of compound IXa giving compounds of general structure IXb.

**Step B:** Upon removal of the benzyl protecting group from the 4-hydroxy moiety of the quinoline by standard hydrogenation methods, quinoline derivatives of general structure IXc are obtained.

#### Synthesis of P3

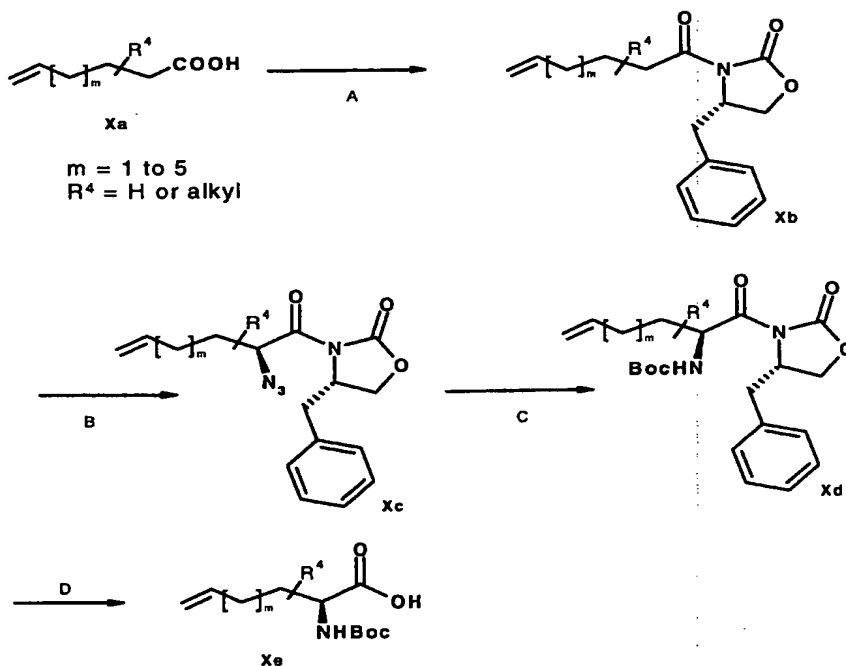
A variety of P3 fragments are synthesized containing the appropriate D linker extension for macrocyclization by olefin metathesis. In general P3 units containing a terminal olefin for metathesis are synthesized following the general schemes shown below (Schemes X, XI & XII).

#### Synthesis of Linkers in Class A

This general synthesis is used to make linkers that are all carbon based (no heteroatom) (Scheme X).

15

Scheme X



The synthesis is performed according to the procedure of Evans *et al.* *J. Am. Chem. Soc.* 1990, 112, 4011-4030.

The starting carboxylic acids (Xa) is commercially available or is prepared by know literature procedures familiar to those skilled in the art.

**Step A:** The carboxylic acid Xa is activated with pivaloyl chloride and then reacted with the anion of Evans' chiral auxiliary 4(S)-4-(phenylmethyl)-2-oxazolidinone  
5 following well known chemistry (Review: D.J. Ager *et al.* *Aldrichimica Acta* 1997, 30, 3-11, and references therein) to obtain compounds of general structure Xb.

**Step B:** Stereoselective  $\alpha$ -azidation with trizylazide, of a chiral imide enolate such as those which would form from compounds with general structure Xb in the presence of a base like KHMDS, is also well known in the art (Review: D.J. Ager *et al.*  
10 *Aldrichimica Acta* 1997, 30, 3-11, and references therein).

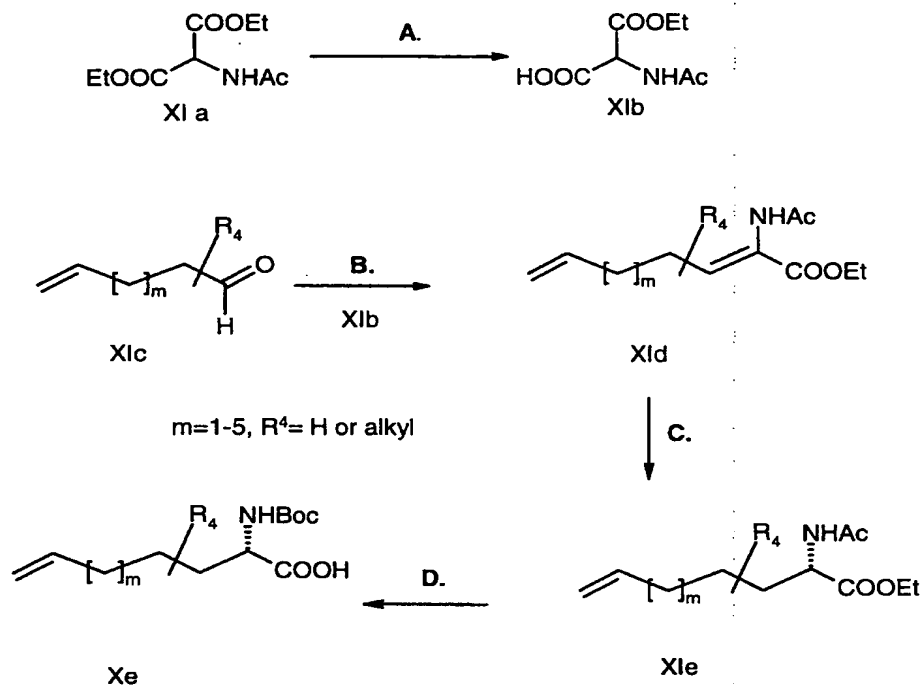
**Step C:** Reduction of the  $\alpha$ -azide, catalyzed by  $\text{SnCl}_2$ , is followed by protection of the amine formed as its t-butyl carbamate gives intermediates of general structure Xc. These reactions are also well known in the art.

**Step D:** Finally, the chiral auxiliary is hydrolyzed under basic conditions, such as a  
15 mixture of  $\text{H}_2\text{O}_2$  with LiOH, to produce the amino acid-type linkers of general structure Xe.

Alternatively, P3 moieties having the same general structure Xe are synthesized following de procedure described by M.J. Burk *et al.* *J. Am. Chem. Soc* 1998, 120, 657-  
20 663 illustrated in Scheme XI. These compounds varied in the number of methylene units ( $-\text{CH}_2-$ ) along the linker ( $m=1$  to 5) and the substitution of alkyl groups at  $\text{R}_4$ , but did not contain a heteroatom.

29

Scheme XI



**Step A:** The monoacid compound XIb is prepared from commercially available diethyl 2-acetamidomalonate by standard ester hydrolysis under basic conditions.

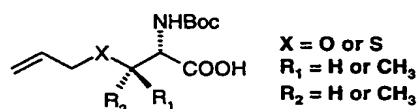
- 5 **Step B:** Knoevenagel-type condensation between an aldehyde of general structure XIc and compound XIb in the presence of a base, such as pyridine, and acetic anhydride leads to the formation of enamide intermediate XId having the Z stereochemistry around the newly formed double bond as shown.

- Step C:** Regioselective and enantioselective catalytic hydrogenation of the enamide intermediate XId to the amino acid intermediate XIe is achieved using Burk's method.

- 10 **Step D:** The nitrogen of the acetamido derivative XIe is then di-protected with the addition of a t-butyl carbamate substituent before the acetate group, as well as the ethyl ester, are hydrolyzed under standard basic condition to obtain P3 moieties of
- 15 general structure XI<sub>f</sub>.

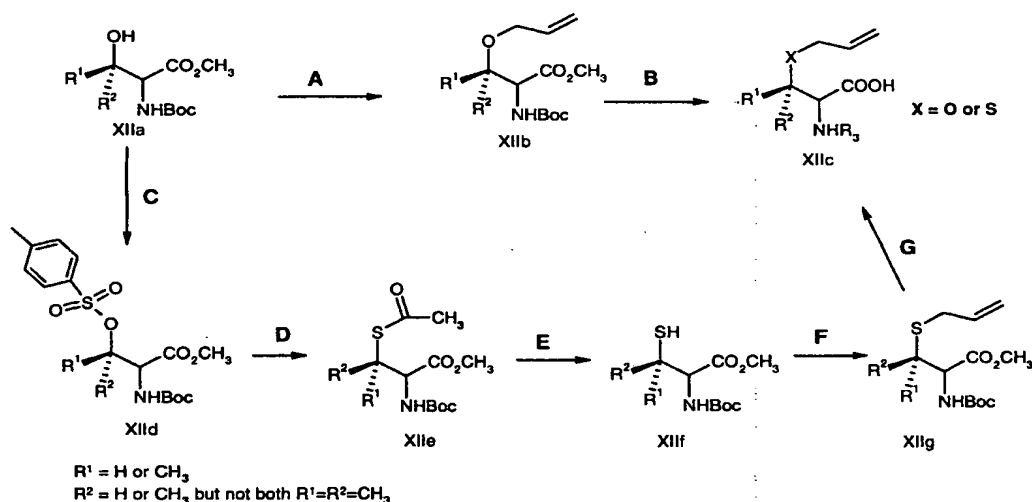
## Synthesis of Linkers in Class B

## General Structure of Linkers in Class B



This general synthesis is used to make linkers containing oxygen or sulfur.

## Scheme XII



5

**Step A:** Suitably protected amino acids, such as Boc-(L)-serine methyl ester, Boc-(L)-threonine methyl ester or Boc-(L)-allothreonine methyl ester, are alkylated with allyl iodide in the presence of  $Ag_2O$  to give the methyl ester XIIb.

**Step B:** Hydrolysis of the methyl ester under standard basic conditions yields the ether-type linkers of general structure XIIc ( $X=O$ ).

**Step C:** The sulfur analog is prepared from the same starting amino acid XIIa (appropriately protected as before) and its hydroxyl group is converted to a good leaving group (such as the tosylate intermediate XIIId) using standard methodology well known in the art.

**Step D:** The tosyl moiety is subsequently displaced with the anion of thioacetate leading to the formation of the thioester intermediate XIIe by inversion of the chiral center at the  $\beta$ -carbon.



## 31

Step E: Hydrolysis of the thioester moiety under mild basic conditions yields the free thiol XIII.

Step F: Alkylation of the thiol moiety is easily achieved under basic conditions with allyl iodide.

- 5 Step G: Finally, the sulfide analog XIIc (X=S) are obtained after hydrolysis of the methyl ester using standard procedures.

**Synthesis of R3 fragment:**

Examples of synthesis of fragments wherein R<sup>3</sup> is NH-R<sup>31</sup> are disclosed in WO 00/09543.

10

**EXAMPLES**

The present invention is illustrated in further detail by the following non-limiting examples. Other specific ways of synthesis or resolution can be found in WO 00/09543 & WO 00/09558.

- 15 Temperatures are given in degrees Celsius. Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer; the chemical shifts ( $\delta$ ) are reported in parts per million and are referenced to the internal deuterated solvent unless otherwise
- 20 indicated. The NMR spectra of all final compounds (inhibitors) was recorded in DMSO-d<sub>6</sub> of their TFA salt unless otherwise indicated. Flash column chromatography was carried out on silica gel (SiO<sub>2</sub>) according to Still's flash chromatography technique (W.C. Still et al., *J. Org. Chem.*, **1978**, *43*, 2923). Abbreviations used in the examples include Bn: benzyl; Boc: *tert*-butoxycarbonyl
- 25 [Me<sub>3</sub>COC(O)]; BSA: bovine serum albumin; Cbz: benzyloxycarbonyl; CHAPS: 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; CH<sub>2</sub>Cl<sub>2</sub>= DCM: methylene chloride; DEAD: diethylazodicarboxylate; DIAD: diisopropylazodicarboxylate; DIPEA: diisopropylethylamine; DMAP: dimethylaminopyridine; DCC: 1,3-
- 30 dicyclohexylcarbodiimide; DME: 1,2-dimethoxyethane; DMF: dimethylformamide; DMSO: dimethylsulfoxide; DTT: dithiothreitol or threo-1,4-dimercapto-2,3-

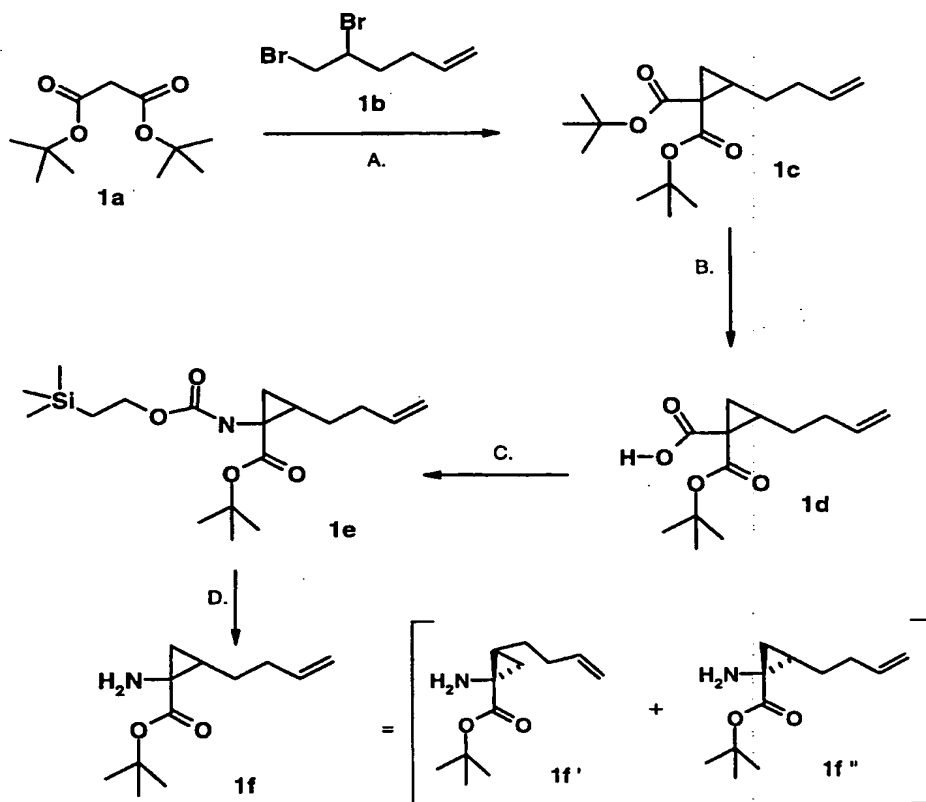
## 32

- butanediol; DPPA: diphenylphosphoryl azide; EDTA: ethylenediaminetetraacetic acid; Et: ethyl; EtOH: ethanol; EtOAc: ethyl acetate; Et<sub>2</sub>O: diethyl ether; ESMS: electrospray mass spectrometry; HATU: O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HPLC: high performance liquid chromatography; MS: mass spectrometry; MALDI-TOF: Matrix Assisted Laser Desorption Ionization-Time of Flight, FAB: Fast Atom Bombardment; LAH: lithium aluminum hydride; Me: methyl; MeOH: methanol; MES: (2-[N-morpholino]ethanesulfonic acid); NaHMDS: sodium bis(trimethylsilyl)amide; NMM: N-methylmorpholine; NMMO: N-methylmorpholine oxide; NMP: N-methylpyrrolidine; Pr: propyl; Succ: 3-carboxypropanoyl; PNA: 4-nitrophenylamino or p-nitroanilide; TBAF: tetra-n-butylammonium fluoride; TBME: *tert*-butyl-methyl ether; *t*BuOK: potassium *tert*-butoxide; TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TCEP: tris(2-carboxyethyl) phosphine hydrochloride; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TIS: triisopropylsilane; TLC: thin layer chromatography; TMSE: trimethylsilylethyl; Tris/HCl: tris(hydroxymethyl)aminomethane hydrochloride.

## P1 MOIETIES

## EXAMPLE 1

Synthesis of *t*-butyl-(1*R*,2*R*)/(1*S*,2*S*)-1-amino-2-homoallylcyclopropyl carboxylate (1f):



- 5 A. To a suspension of benzyltriethylammonium chloride (5.08 g, 22.3 mmol.) in 50% aqueous NaOH (50 mL), 1,2-dibromo-5-hexene (1b, 8.10 g, 33.46 mmol) and di-*t*-butylmalonate (1a, 4.82 g, 22.30 mmol) were added in succession. The mixture was stirred vigorously at RT for 16 h, then diluted with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 50 mL). The organic layer was further washed with H<sub>2</sub>O (2 x 50 mL), brine/H<sub>2</sub>O (2/1, 2 x 50 mL, dried over MgSO<sub>4</sub> and evaporated. The crude residue was purified by flash column chromatography on silica gel, using 3 to 5% EtOAc in hexane as the eluent, to obtain compound 1c in 38% yield (2.48 g).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.19 (bd, J = 7.9 Hz, 2H), 1.25-1.33 (m, 1H), 1.46 (s, 9H),

1.48 (s, 9H), 1.47-1.60 (m, 1H), 1.75-1.82 (m, 1H), 2.14-2.22 (m, 2H), 4.93-5.50 (m, 2H), 4.96 (dm,  $J = 10.2$  Hz, 1H), 5.18 (dm,  $J = 17.2$  Hz, 1H). ES(+)MS  $m/z$  297 ( $M+H$ )<sup>+</sup>.

B. To a suspension of potassium *t*-butoxide (5.75 g, 51.25 mmol) in anhydrous diethyl ether (150 mL) at 0°, H<sub>2</sub>O was added (203  $\mu$ L, 11.27 mmol) and the reaction mixture was stirred at 0° for 10 min. An ether solution of compound 1c (2.48 g in 10 mL diethyl ether, 10.25 mmol) was added and the mixture was stirred at RT for 5 h. The mixture was diluted with ice-cold H<sub>2</sub>O and extracted with diethyl ether (3 x 200 mL). The aqueous layer was acidified to pH 3.5-4 with ice-cold 10% aqueous citric acid and re-extracted with EtOAc (3 x 200 mL). The EtOAc layer was washed with H<sub>2</sub>O (2 x 100 mL), brine (100 mL), dried over MgSO<sub>4</sub> and evaporated to give compound 1d in 85% yield based on the amount of recovered starting material.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.51 (s, 9H), 1.64-1.68 (m, 1H), 1.68-1.75 (m, 1H), 1.77-1.88 (m, 1H), 1.96-2.01 (m, 1H), 2.03-2.22 (m, 3H), 5.01 (dm,  $J = 6.4$  Hz, 1H), 5.03 (dm,  $J = 14.9$  Hz, 1H), 5.72-5.83 (m, 1H).

ES(+)MS:  $m/z$  241 ( $M+H$ )<sup>+</sup>.

C. To a solution of the acid 1d in anhydrous benzene (1.14 g in 25 mL benzene, 4.74 mmol), Et<sub>3</sub>N (800  $\mu$ L, 5.68 mmol) was added, followed by the addition of diphenylphosphoryl azide (1.13 mL, 5.21 mmol) and the mixture was heated to reflux for 3.5 h. Subsequently, trimethylsilylethanol (1.36 mL, 9.48 mmol) was added and stirring at reflux was continued for an additional 4 h. The mixture was then cooled to RT, evaporated to half of its original volume, diluted with diethyl ether (30 mL) and washed with 5% aqueous NaHCO<sub>3</sub> (2 x 30 mL), brine (50 mL), dried over MgSO<sub>4</sub> and evaporated. The residual oil was chromatographed on silica gel using 10% EtOAc in hexane as the eluent to obtain pure compound 1e in 88% yield (1.49 g).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.03 (s, 9H), 0.91-0.99 (m, 2H), 1.18-1.29 (m, 2H), 1.45 (bs, 11H), 1.56-1.72 (m, 2H), 2.02-2.18 (m, 2H), 4.12 (t,  $J = 8.3$  Hz, 2H), 4.93 (dm,  $J = 10.2$  Hz, 1H), 4.98 (dm,  $J = 17.2$  Hz, 1H), 5.07 (bs, 1H), 5.71-5.83 (m, 1H).

D. To a solution of the cyclopropyl derivative 1e (1.19 g, 3.35 mmol, in 30 mL

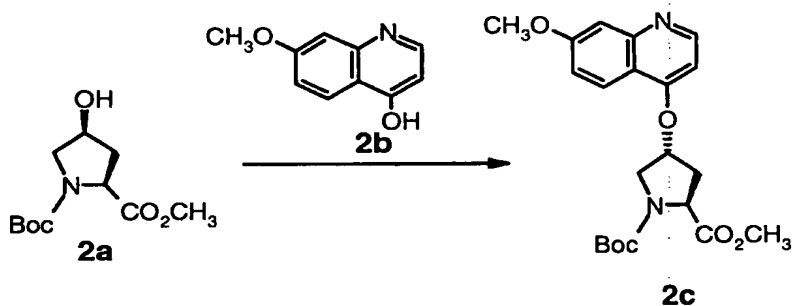
## 35

THF),  $t\text{-Bu}_4\text{NF}$  (6.7 mL of 1M in THF, 6.7 mmol.) was added and the mixture was first stirred at RT for 16 h and subsequently heated to reflux for 15 min. The solvent was carefully evaporated under low pressure (due to the high volatility of the free amine **1f**, caution should be exercised during the evaporation of the solvent). The crude residue was re-dissolved in EtOAc (100 mL) and washed with  $\text{H}_2\text{O}$  (2x 50mL),  
 5 brine (50mL), dried over  $\text{MgSO}_4$  and again the solvent was carefully evaporated. The crude product **1f** ( as a mixture of two enantiomers **1f'** and **1f''**) was used for coupling with the P2 proline derivatives without further purification. Isolation of the P1P2 fragment having the desired stereochemistry at P1 was easily achieved at this  
 10 stage using flash chromatography (example 21, fragment **21b**).

## P2 MOIETIES

## EXAMPLE 2

Synthesis of Boc-4(R)-[(7-methoxy-4-quinolinyloxy)proline (**2c**):



15

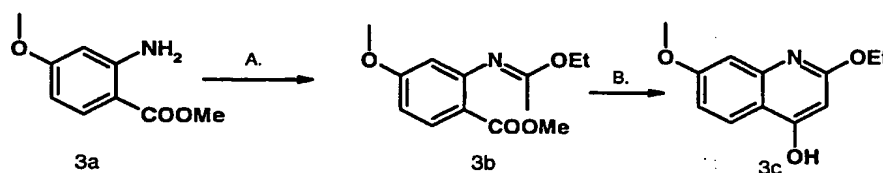
4-Hydroxy-7-methoxyquinoline (**2b**) was prepared according to the method described by Chun, M.W.; Olmstead, K.K.; Choi, Y.S.; Lee, C.O.; Lee, C.-K.; Kim, J.H.; Lee, *J. Bioorg. Med. Chem. Lett.* 1997, 7, 789. A solution of compound **2b** (1.88 g, 10.73 mmol) and DEAD (3.4 mL, 21.46 mmol) in anhydrous THF were added to a stirring solution of protected *cis*-hydroxyproline **2a** (2.63 g, 10.73 mmol) and triphenylphosphine (5.63 g, 21.46 mmol) in anhydrous THF (160 mL) at 0° under  $\text{N}_2$ . The reaction mixture was allowed to warm-up to RT and stir for 14 h. The THF was then evaporated and the pure product **2c** was isolated after flash column chromatography using 5% MeOH in EtOAc as the eluent, in 35% yield (1.5 g).

25  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.44 (s, 9H), 1.65 (bs, 1H), 2.34-2.43 (m, 1H), 2.63-2.76

(m, 1H), 3.78 (s, 3H), 3.75-3.85 & 3.89-3.99 (2m, 1H, 2 rotamers), 3.95 (s, 3H), 4.51 & 4.60 (2t, J = 8 Hz, 1H, 2 rotamers), 5.15 (bs, 1H), 6.53-6.59 (m, 1H), 7.12-7.18 (dd, J = 8.9 & 2.2 Hz, 1H), 7.36 (d, J = 2.6 Hz, 1H), 8.03 (bd, J = 9.2 Hz, 1H), 8.65 (bd, J = 5.1 Hz, 1H).

5    **EXAMPLE 3**

**Synthesis of 2-ethoxy-4-hydroxy-7-methoxy quinoline (3c)**



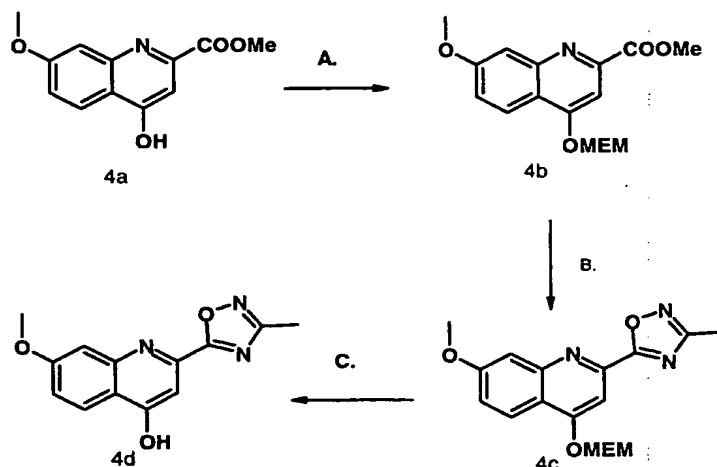
The synthesis of Methyl-*p*-methoxyantranilate 3a was done as described in Katz *et al. J. Org. Chem.*, 1953, 18, 1380-1400.

- 10    The general synthesis for the quinoline derivative 3c is a modification of the procedure of Baccar *et al. Indian Journal of Chemistry*, 1995, *Sat. B*, 330-332.

A.    Methyl-*p*-methoxyantranilate 3a (3.069 g, 16.96 mmol) was dissolved in triethylorthoacetate (4.7 mL, 25.4 mmol), then a solution of anhydrous HCl (4 N/Dioxane, 50  $\mu$ L, 0.6 mmol) was added. The resulting mixture was heated at  
15    reflux for 19 hours. The volatiles were then evaporated under vacuum to give product 3b (4.92 g, amber oil, quantitative yield) that was used as such for the next step.

B.    To a solution of the substrate 3b (assumed 16.96 mmol) in THF (34 mL) at -78°C under nitrogen, was added LiHMDS (1 M/THF, 22 mL, 1.3 eq.). Shortly after  
20    the addition, the cold temperature bath was removed and the mixture was left to stir at ambient temperature for 1 hour, after which time, another portion of LiHMDS (16 mL) was added. The resulting mixture was stirred until complete disappearance of starting material (1 hour) by TLC (100% EtOAc, imidate  $R_f$  = 0.7, product  $R_f$  = 0.2).  
HCl (4 N/dioxane, 10 mL) was then added and the mixture was concentrated under  
25    vacuum. The resulting paste was triturated from a mixture of EtOAc (10 mL) and aqueous  $\text{NaH}_2\text{PO}_4$  (1 M, 10 mL) and sonicated. An abundant precipitate was formed, collected by filtration, washed with water and dried to afford the desired product 3c as a beige solid (3.117 g, 84% yield for 2 steps, >99% purity by HPLC).

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 7.88 (d,  $J$  = 8.9 Hz, 1H), 6.98 (br. s, 1H), 6.89 (br. d,  $J$  = 8.6 Hz, 1H), 5.94 (br. s, 1H), 4.30 (br. s, 2H), 3.84 (s, 3H), 1.34 (t,  $J$  = 7.0 Hz, 3H).

**EXAMPLE 4****5 Synthesis of 4-hydroxy-7-methoxy-2(3-methyl-1,2,4-oxadiazol-5-yl) quinoline (4d)**

A. To a solution of 2-carbomethoxy-4-hydroxy-7-methoxyquinoline 4a (the preparation of which is described in WO 00/09543 and WO 00/09558) (1 g, 4.29 mmol) in DMF (10 mL) under nitrogen was added NaH (60% in mineral oil, 190 mg, 4.98 mmol). The resulting mixture was stirred at ambient temperature for 1 hour, MEM chloride (455  $\mu\text{L}$ , 4.98 mmol) was then added dropwise and the resulting mixture was stirred at ambient temperature for an extra 19.5 hours. The reaction mixture was diluted with EtOAc (100 mL), washed with  $\text{H}_2\text{O}$  (50 mL), brine (50 mL), dried with  $\text{MgSO}_4$ , concentrated under vacuum to afford the crude reaction isolate (1.37 g). The latter was purified by flash column chromatography to afford product 4b (1.04 g, 75% yield) as a colorless oil.

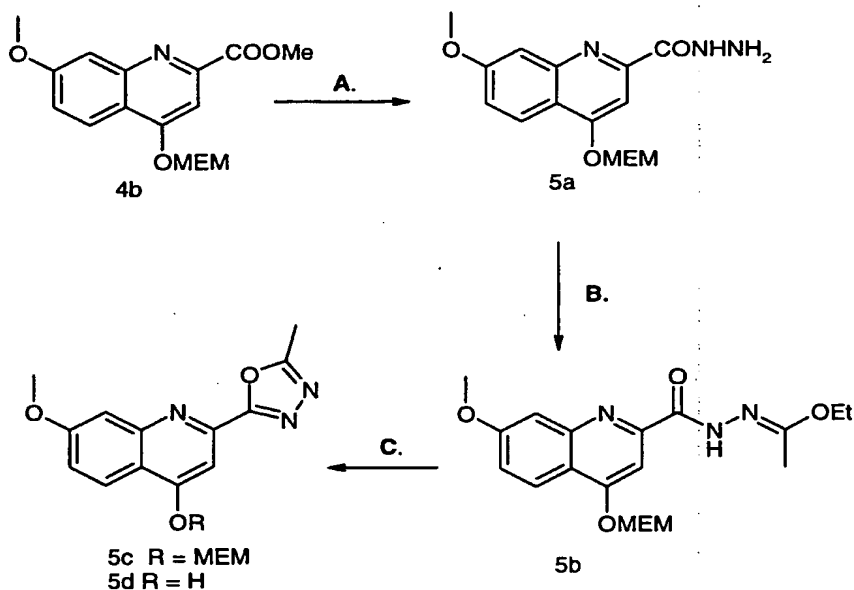
B. To a mixture of freshly activated 4Å molecular sieve (500 mg) and acetamidoxime (248 mg, 3.35 mmol) was added THF (3mL). The resulting mixture was stirred for 15 min. under nitrogen at ambient temperature, then NaH (60% in mineral oil, 124 mg, 3.24 mmol) was added by portions. The resulting suspension was stirred at ambient temperature for 1 hour, then ester 4b (500 mg, 1.56 mmol) was added in solution in THF (5 mL). The resulting mixture was heated at reflux for

1 hour then filtered over Celite, rinsing with EtOAc (3 portions of 20 mL) and concentrated under vacuum. The resulting crude mixture was purified by flash column chromatography (100% EtOAc) to afford product **4c** (352 mg, 65% yield) as a white solid.

- 5 C. To the MEM ether **4c** (170 mg, 0.493 mmol) in THF (4 mL) was added aqueous HCl (1 N, 1 mL). The resulting mixture was stirred at ambient temperature for 1 hour then diluted with aqueous  $\text{NaH}_2\text{PO}_4$  (1 M, 50 mL). The solid formed was filtered, triturated with EtOAc, filtered and dried to afford the desired product (**4d**) (90 mg, 71% yield) as a white solid. MS (ES+) 258 (M+1), (ES-) 256 (M-1).
- 10  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.03 (d,  $J = 9.2$  Hz, 1H), 7.38 (d,  $J = 2.2$  Hz, 1H), 7.06 (d,  $J = 8.6$  Hz, 1H), 6.85 (br. s, 1H), 3.88 (s, 3H), 2.64 (s, 3H).

#### EXAMPLE 5

Synthesis of 4-hydroxy-7-methoxy-2(5-methyl-1,3,4-oxadiazol-2-yl) quinoline (**5e**)



- 15 A. To substrate **4b** (465 mg, 1.45 mmol) in ethanol (5 mL) was added anhydrous hydrazine (57  $\mu\text{L}$ , 1.8 mmol). The resulting solution was heated at reflux for 4 h, then concentrated under vacuum to afford product **5a** (704 mg, quantitative crude yield) as a yellow solid which was used as such in the next step.
- B. Compound **5a** (assumed 1.45 mmol) in triethylorthoacetate (5 mL) was

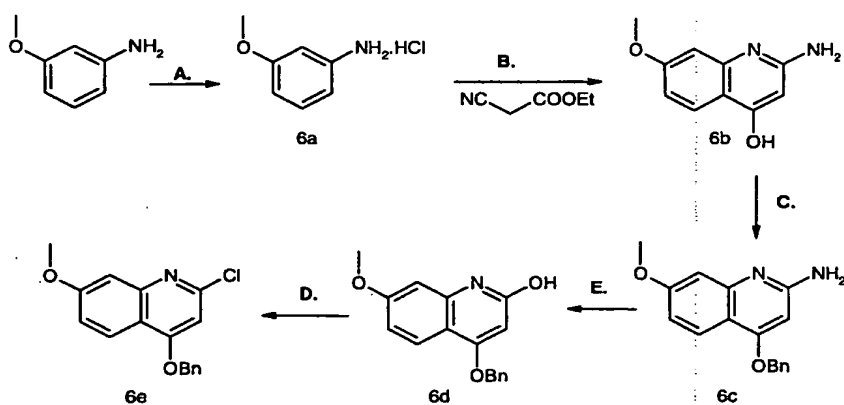


heated at 100-110 °C under nitrogen. The resulting mixture was then diluted with EtOAc (100 mL), washed with aqueous saturated NaHCO<sub>3</sub> (50 mL), brine (50 mL), dried with MgSO<sub>4</sub>, concentrated under vacuum and purified by flash column chromatography (100% EtOAc). Compound 5b (359 mg, 61% yield for two steps) was obtained as a yellow oil. MS (ES+) 392 (m+1), (ES-) 390 (m-1).

C. Compound 5b (333 mg, 0.852 mmol) was heated at 140 °C under high vacuum for 8.5 h and purified by flash column chromatography (100% EtOAc) to afford a mixture of 5b (116 mg, 35%, R<sub>f</sub> 0.5) and compound 5c (138 mg, 72% corrected yield, R<sub>f</sub> 0.3). To a THF (4 mL) solution of compound 5c (138 mg, 0.4 mmol) was added aqueous HCl (1 N, 1 mL) and the resulting mixture was stirred until completion (30 min.). THF was evaporated under vacuum and aqueous NaH<sub>2</sub>PO<sub>4</sub> (1 M, 2 mL) was added. The resulting suspension was sonicated, filtered and the solid was dried under high vacuum to afford the desired product 5d, (75 mg, 73% yield) as a beige solid. MS (ES+) 258 (m+1), (ES-) 256 (m-1). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.03 (d, J = 9.2 Hz, 1H), 7.39 (d, J = 2.2 Hz, 1H), 7.06 (br. d, J = 8.6 Hz, 1H), 6.85 (br. s, 1H), 3.88 (s, 3H), 2.64 (s, 3H).

#### EXAMPLE 6

##### Synthesis of 4-benzyloxy-2-(chloro)-7-methoxyquinoline (6e)



A. Commercially available *Meta*-anisidine (25 g, 0.20 mol) in dioxane (80 mL) was cooled down to 0 °C and anhydrous HCl (4 N/dioxane, 75 mL, 0.30 mol) was added. Then Et<sub>2</sub>O (500 mL) was added and stirring was maintained for 1 hour. The

beige solid was then filtered and dried under vacuum to afford salt 6a (31.88 g, 98% yield).

B. To this salt was added ethylcyanoacetate (21.3 mL, 0.20 mol) and the mixture, in a flask equipped with a distillation head and a collecting flask, was heated to 280-300 °C. Ethanol produced was collected to monitor the evolution of the reaction. At 9 mL of collected ethanol (theoretical amount 11.7 mL), heating was stopped, the reaction mixture cooled down to RT, diluted with water (200 mL) – EtOAc (200 mL) then stirred and aqueous  $\text{NaH}_2\text{PO}_4$  (300 mL) was added. After additional stirring for 1 h, filtration and drying, 6b was obtained (19.06 g, 84.5% purity, ~50% yield) as a yellow solid and was used as such in the next reaction.

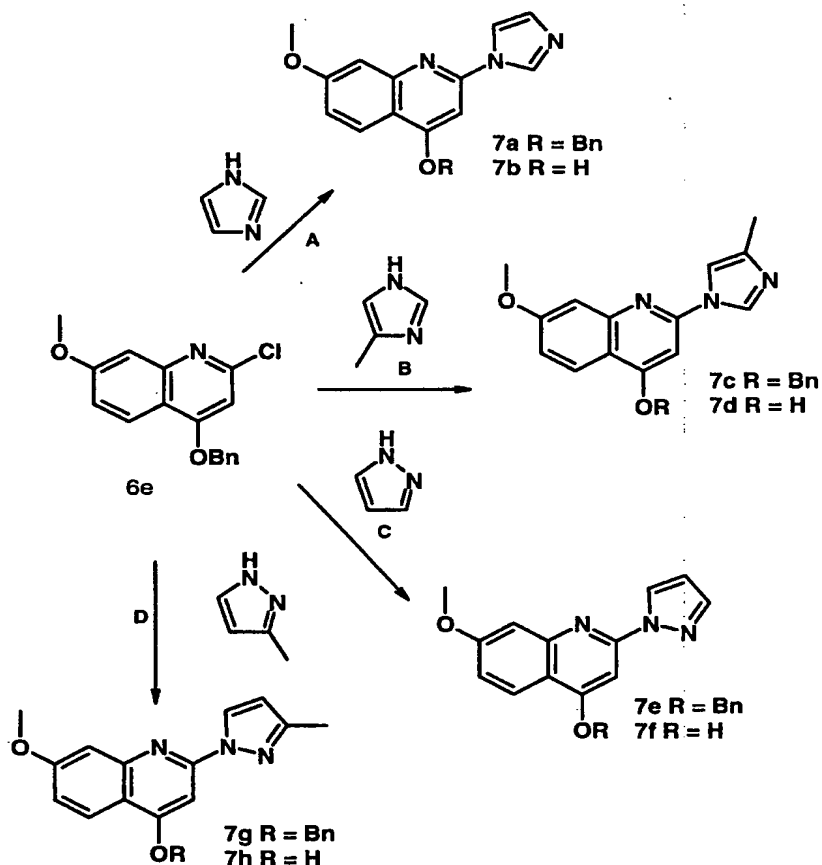
C. Compound 6b (11.0 g, 57.8 mmol) in DMF (100 mL) at 0 °C was added to NaH (60% in mineral oil, 2.78 g, 115.6 mmol). The ice bath was then removed and the mixture was stirred at ambient temperature for 1 h, benzyl bromide (7.6 mL, 63.6 mmol) was then added and the reaction mixture was stirred for 16 hours. The solution was then diluted with EtOAc (220 mL) – hexane (220 mL) and the solid formed was filtered, triturated with aqueous saturated  $\text{NaHCO}_3$  (110 mL), washed with water, hexane–EtOAc (1:1 ratio, 100 mL) and dried under high vacuum. Product 6c (5.6 g, 91% purity, 35% yield) was thus obtained as a yellow solid. To compound 6c (2.67 g, 9.52 mmol) in acetic acid (21 mL) was added *iso*-amyl nitrite (3.8 mL, 28.6 mmol) and the resulting mixture was stirred at ambient temperature and monitored by HPLC. More *iso*-amyl nitrite (1.3 mL, 9.52 mmol) was added after 2 hours and the mixture was left to stir over 90 hours (HPLC 81% product, 3% substrate). Water (100 mL) was added to the resulting suspension, which was then filtered. The brown solid collected was dried under high vacuum giving product 6d (2.35 g, 92% purity, 72% yield).

D. To compound 6d (1.5 g, 4.39 mmol) was added phosphorous oxychloride (13 mL, 141 mmol) and the resulting mixture was heated at reflux for 1 hour then diluted with EtOAc (150 mL) and quenched at 0 °C slowly with aqueous NaOH (1 N, 150 mL) to pH 9. The two layers were separated and the organic layer was dried with  $\text{MgSO}_4$  and concentrated under vacuum to afford a brown solid which was purified by flash column chromatography (15 % EtOAc/hexane). Product 6e (819 mg, purity > 99%, 62% yield) was obtained as a yellow solid.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.07 (d,  $J$  = 9.2 Hz, 1H), 7.50-7.40 (m, 5H), 7.29 (d,  $J$  = 2.5 Hz, 1H), 7.12 (dd,  $J$  = 9.2, 2.5 Hz, 1H), 6.73 (s, 1H), 5.26 (s, 2H), 3.92 (s, 3H).

#### EXAMPLE 7

- Synthesis of 4-hydroxy-2-(1-imidazolyl)-7-methoxyquinoline (7b); 4-hydroxy-2-(4-methyl-1-imidazolyl)-7-methoxyquinoline (7d); 4-hydroxy-7-methoxy-2-(1-pyrazolyl)quinoline (7f); and 4-hydroxy-2-(3-methyl-1-pyrazolyl)-7-methoxyquinoline (7h).



- A. Compound 6e (423 mg, 1.41 mmol) and imidazole (400 mg, 5.88 mmol.) were heated at 110 °C for 20 h. The mixture was then diluted with EtOAc and washed with water and brine, dried with  $\text{MgSO}_4$ , concentrated under reduced pressure to afford compound 7a (422 mg, 96% purity, 90% yield) as a yellow solid. Compound 7a (319 mg, 0.963 mmol) with Pd (5%/C, 64 mg) in a mixture of ethanol (5 mL) and

THF (5 mL) was purged and placed under one ATM. of hydrogen. After 7.5 h of stirring at ambient temperature, the reaction mixture was filtered, rinsed with a chloroform-methanol mixture, and concentrated to afford 7b (130 mg, 97.7% purity, 56% yield) as a yellow solid. MS (ES+) 242 (m+1), (ES-) 240 (m-1).

- 5 <sup>1</sup>H NMR (400 MHz, DMSO-d): δ 8.51 (s, 1H), 8.03 (d, J = 8.9 Hz, 1H), 7.93 (s, 1H), 7.23 (d, J = 1.9 Hz, 1H), 7.15 (s, 1H), 7.12 (dd, J = 9.2, 2.2 Hz, 1H), 6.92 (br. s, 1H), 3.91 (s, 3H).

- B. Compound 6e (251 mg, 0.837 mmol) and 4-methylimidazole (344 mg, 4.19 mmol.) were heated at 110 °C for 20 h. The mixture was then diluted with EtOAc, washed with water and brine, dried with MgSO<sub>4</sub>, and concentrated under reduced pressure to afford a crude containing a 10:1 mixture of 4-methyl and 5-methylimidazolyl isomer respectively. The major assumed desired isomer 11c, a white solid, (166 mg, 99% purity, 57% yield) was separated from a second more polar fraction (76 mg, 23% yield) containing a mixture of 4- and 5-methyl imidazolyl isomer by flash column chromatography (100% EtOAc). Compound 7c (163 mg, 0.472 mmol) with Pd (5%/C, 33 mg) in a mixture of ethanol (2.4 mL) and THF (5 mL) was purged and placed under one ATM. of hydrogen. After 18 h of stirring at ambient temperature, the reaction mixture was filtered, rinsed with a chloroform-methanol mixture, and concentrated to afford 7d (118 mg, 99% purity, 98% yield) as a white solid.
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<sup>1</sup>H NMR (400 MHz, DMSO-d): δ 8.42 (br. s, 1H), 8.01 (d, J = 9.2 Hz, 1H), 7.64 (br. s, 1H), 7.21 (br. s, 1H), 7.10 (d, J = 8.9 Hz, 1H), 6.89 (br. s, 1H), 3.90 (s, 3H), 2.20 (s, 3H).

- C. Compound 6e (184 mg, 0.614 mmol) and pyrazole (209 mg, 3.07 mmol.) were heated at 110 °C for 17 h. The mixture was then diluted with EtOAc and washed with aqueous NaOH (1 N) and brine, dried with MgSO<sub>4</sub>, concentrated under reduced pressure to afford a crude product which was purified by flash column chromatography (2 : 1 hexane-EtOAc) to afford 7e (103 mg, 50% yield) as a pale yellow solid. Compound 7e (103 mg, 0.311 mmol) with Pd (5%/C, 20 mg) in a mixture of ethanol (2 mL) and THF (2 mL) was purged and placed under one atm. of hydrogen. After 5.5 h of stirring at ambient temperature, the reaction mixture was filtered, rinsed with a chloroform-methanol mixture, and concentrated to afford 7f
- 25  
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(77 mg, 99% purity, 99% yield) as a yellow solid. MS (ES+) 242 (m+1), (ES-) 240 (m-1).

<sup>1</sup>H NMR (400 MHz, DMSO-d): δ 8.72 (d, J = 2.5 Hz, 1H), 8.31 (s, 1H), 8.00 (d, J = 8.9 Hz, 1H), 7.83 (br. s, 1H), 7.43 (br. s, 1H), 7.24 (br. s, 1H), 7.10 (d, J = 8.6 Hz, 1H), 6.59 (br. s, 1H), 3.90 (s, 3H).

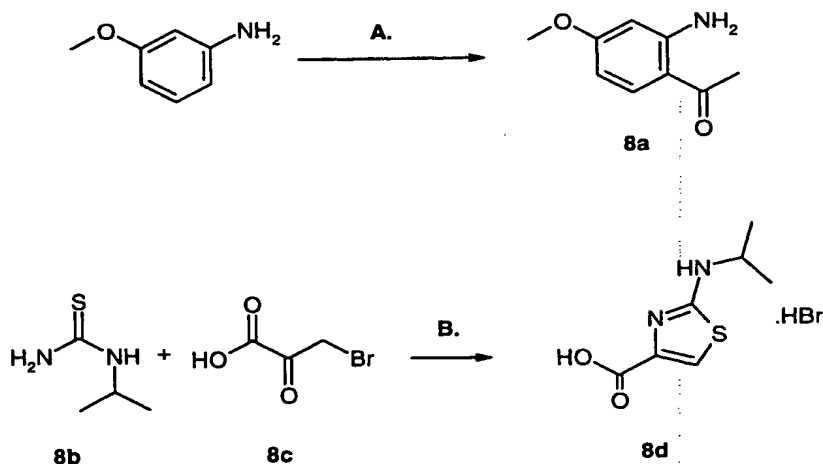
D. Compound 6e (217 mg, 0.724 mmol) and 4-methylpyrazole (594 mg, 7.24 mmol.) were heated at 110 °C for 23 h. The mixture showing a 1 : 1 mixture of debenzylated compound 7h and benzylated product 7g was then diluted with EtOAc (2-3 mL) and filtered to afford the pure debenzylated product 7h (111 mg, 95% purity, 54% yield) as a white solid.

<sup>1</sup>H NMR (400 MHz, DMSO-d): δ 8.58 (d, J = 2.6 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.25 (br. s, 1H), 7.20 (s, 1H), 7.04 (br. d, J = 9.2 Hz, 1H), 6.38 (s, 1H), 3.89 (s, 3H), 2.30 (s, 3H).

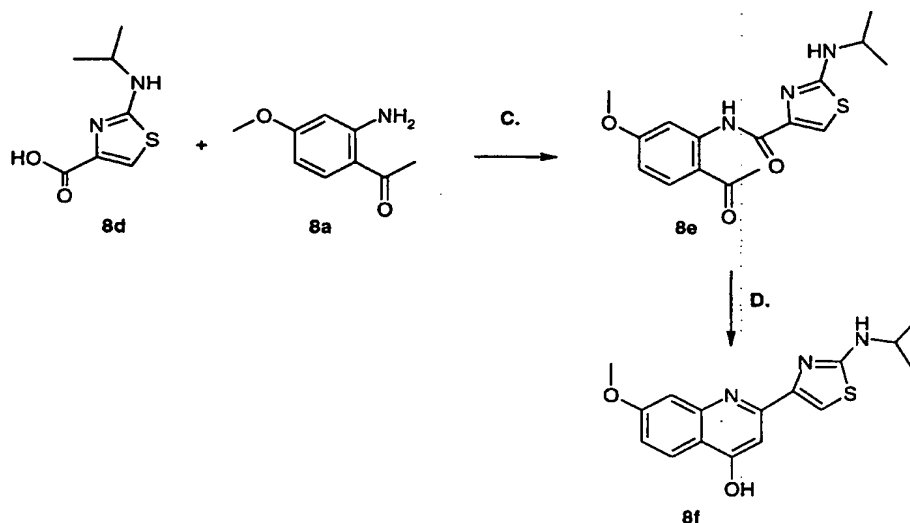
#### EXAMPLE 8

#### 15 Synthesis of 4-hydroxy-7-methoxy-2[4(2-isopropylaminothiazolyl)] quinoline (8f)

Note: [ A variety of 2-alkylaminothiazolyl substituents were made using the same synthetic scheme where compound 8b was replaced by other alkyl thioureas.]



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- A. The protocol used for the conversion of *m*-anisidine to 8a was identical to that described in the literature: F.J. Brown *et al. J. Med. Chem.* **1989**, *32*, 807-826. However, the purification procedure was modified to avoid purification by chromatography. The EtOAc phase containing the desired product was treated with a mixture of MgSO<sub>4</sub>, charcoal and 5% w/w (based on expected mass) silica gel. After filtration on celite, the product was triturated with ether. Compound 8a was obtained as a pale brown solid in >99% purity (as confirmed by HPLC).
- B. A suspension of isopropyl thiourea (8b, 3.55 g, 30 mmol) and 3-bromopyruvic acid (8c, 5 g, 1 eq.) in dioxane (300 mL, 0.1 M) was heated to 80 °C. Upon reaching 80 °C the solution became clear and soon after the product precipitated as a white solid. After 2 hours of heating, the solution was cooled to RT and the white precipitate was filtered to obtain compound 8d in high purity (>98% purity as confirmed by NMR) and 94% yield (7.51 g).
- C. A mixture of the carboxylic acid 8d (4.85 g, 18.2 mmol) and the aniline derivative 8a (3 g, 1eq.) in pyridine (150 mL, 0.12 M) was cooled to -30 °C (upon cooling, the clear solution became partially a suspension). Phosphorus oxychloride (3.56 mL, 2.1 eq.) was then added slowly over a 5 min period. The reaction was stirred at -30 °C for 1 h, the bath was removed and the reaction mixture was allowed to warm-up to RT. After 1.5 h the reaction mixture was poured into ice, the pH was

adjusted to 11 with aqueous 3N NaOH, extracted with CH<sub>2</sub>Cl<sub>2</sub>, dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated under vacuum. The beige solid was then purified by flash chromatography (45% EtOAc in hexane) to give compound 8e as a pale yellow solid in 73% yield (6.07 g).

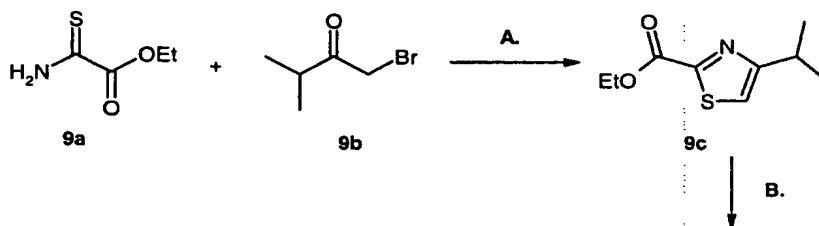
- 5 D. A solution of tBuOK (2.42 g, 21.6 mmol) in anhydrous tBuOH (40ml, 0.14 M, distilled from Mg metal) was heated to reflux. Compound 8e (1.8g, 5.4 mmol) was added portion-wise over 5 min and the dark red solution formed was stirred at reflux for an additional 20 min (completion of the reaction was monitored by HPLC). The mixture was cooled to RT and HCl was added (4 N in dioxane, 1.5 eq.).
- 10 The mixture was then concentrated under vacuum, in order to assure that all of the HCl and dioxane were removed, the product was re-dissolved twice in CH<sub>2</sub>Cl<sub>2</sub> and dried under vacuum to finally obtain the HCl salt of compound 8f as a beige solid (1.62 g, 93% pure by HPLC). The product was then poured into a phosphate buffer (1N NaH<sub>2</sub>PO<sub>4</sub>, pH=4.5) and sonicated. The beige solid was filtered and dried under
- 15 vacuum to give compound 8f (1.38 g, 81% yield) as a beige solid (91% pure by HPLC).

<sup>1</sup>H NMR (400 MHz, DMSO) δ 8.27 (s, 1H), 8.12 (d, 1H, J = 9.2 Hz), 7.97 (br.s, 1H), 7.94 (s, 1H), 7.43 (s, 1H), 7.24 (dd, 1H, J = 9.2, 2.2 Hz), 3.97 (m, 1H), 3.94 (s, 3H), 1.24 (d, 2H, J = 6.4 Hz).

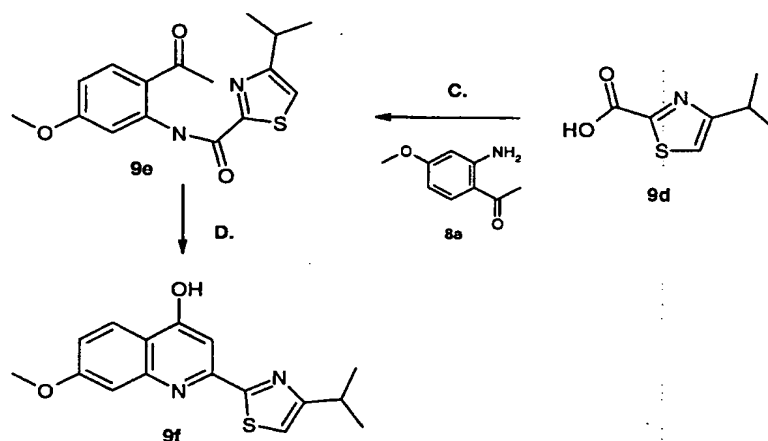
20 **EXAMPLE 9**

**Synthesis of 4-hydroxy-7-methoxy-2[2(4-isopropylthiazolyl)]quinoline (9f).**

**Note:** A variety of 2-(4-alkyl)-thiazolyl substituents were made using the same synthetic scheme where compound 9b was replaced by other α-bromoketones.



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- A. To a solution of 3-methyl-butan-2-one (8 g, 93 mmol) in MeOH (100 mL) at  $-30^{\circ}\text{C}$ ,  $\text{Br}_2$  (4.79 mL, 93 mmol, 1 eq.) was added dropwise over a period of 45 min. The resulting mixture was then stirred at RT for 90 min. Pentane was added and the solution washed with 5% aqueous  $\text{NaHCO}_3$ , the organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under vacuum. The resulting crude yellow oil, compound 9b, was used without further purification. A solution of ethyl thiooxamate (9a, 1.8 g, 13.5 mmol) and bromoketone derivative 9b (13.5 mmol.) was stirred at  $70^{\circ}\text{C}$  for 15 h. The mixture was then concentrated under vacuum and subsequently purified by flash column chromatography, using 15% EtOAc in hexane as the eluent, to obtain compound 9c (740 mg, 28 % yield).
- B. A solution of compound 9c (700 mg, 3.5 mmol) in THF/MeOH/ $\text{H}_2\text{O}$  (3:1:1 ratio, 13 mL) was treated with  $\text{LiOH}\cdot\text{H}_2\text{O}$  (148 mg, 3.5 mmol, 1eq.) at RT for 5 h. The pH was then adjusted to 6 with 0.1N HCl and the mixture was concentrated to dryness under vacuum to obtain the acid 13d, which was used directly in the next step without further purification.
- C. A solution of 4-methoxy-2-amino-acetophenone (intermediate 8a, 570 mg, 3.45 mmol) and carboxylic acid derivative 9d (590 mg, 3.45 mmol, 1eq.) in pyridine (30 mL) was cooled to  $-20^{\circ}\text{C}$ .  $\text{POCl}_3$  (0.35 mL, 3.79 mmol, 1.1 eq.) was then added dropwise over a period of 5 min. The resulting solution was stirred at  $-10^{\circ}\text{C}$  for 2 h. The reaction was quenched with the addition of  $\text{H}_2\text{O}$  and the mixture was

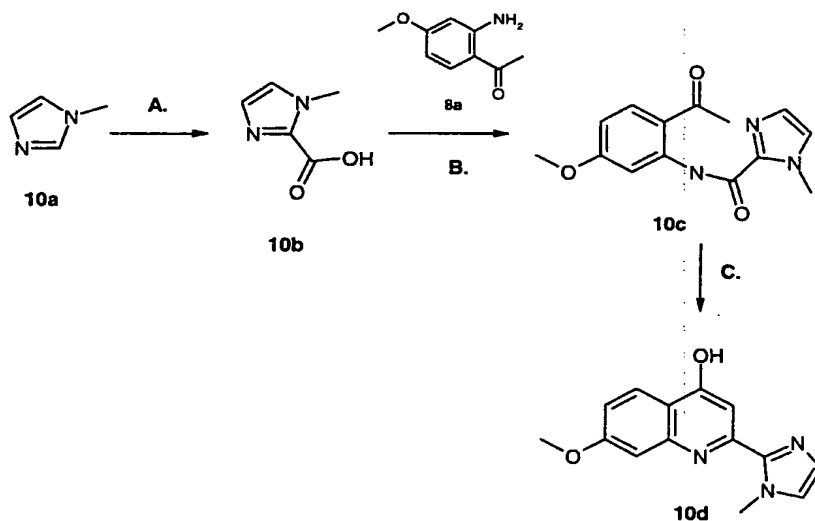


concentrated under vacuum. The residue was poured in a saturated aqueous solution of  $\text{NaHCO}_3$  and extracted with EtOAc. The organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated under vacuum. The crude product was purified by flash column chromatography, using 25%EtOAc in hexane as the eluent, to give compound 9e as a white solid (740 mg, 67% yield).

D. tBuOK (518 mg, 2.1 eq.) was added to a suspension of compound 9e (700 mg, 2.2 mmol) in anhydrous tBuOH (11 mL). The resulting mixture was heated to 75 °C for 7.5 h, the solution was then cooled to RT and acidified with the addition of HCl (4N HCl in dioxane, 2.5 mL). The mixture was concentrated under vacuum and the residue obtained was poured into a solution of 1N  $\text{NaH}_2\text{PO}_4$  and filtered. The solid material was then triturated with a small amount of EtOAc, filtered and dried under vacuum to obtain compound 9f as a pale beige solid (270 mg, 41% yield).  
 $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.00 (br. s, 1H), 7.60 (br. s, 1H), 7.51 (br. s, 1H), 7.43 (br. s, 1H), 7.29 (br. s, 1H), 7.14 (br. s, 1H), 6.95 (br. a, 1H), 3.90 (s, 3H), 3.15 (m, 1H), 1.33 (d,  $J=5.4$  Hz, 6H).

#### EXAMPLE 10

##### Synthesis of 4-hydroxy-2-(1-methyl-2-imidazolyl)-7-methoxyquinoline (10d)

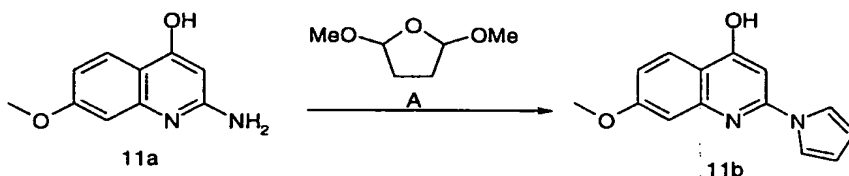


A. A solution of N-methylimidazole 10a (5g, 61 mmol) in 100 mL THF was cooled at -78° C. n-BuLi (24.4 ml of a 2.5M/ $\text{Et}_2\text{O}$  solution, 1 eq.) was added

dropwise over 15 min. The resulting mixture was stirred 90 min. at -78° C then poured portionwise over excess solid CO<sub>2</sub>. The heterogeneous mixture was stirred 2 h and allowed to reach RT. 1N HCl was added to pH 5, the aqueous layer is separated and lyophilized. The residue thus obtained was extracted with EtOAc (to  
5 remove salts), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. 6.2 g (80% yield) of a white solid **10b** was obtained.

B. A solution of 4-methoxy-2-amino-acetophenone **8a** (394 mg, 2.39 mmol) and the carboxylic acid derivative **10b** (301 mg, 1eq.) in pyridine (10 ml) was cooled to -20° C. POCl<sub>3</sub> (244 µl, 1.1 eq.) was then added dropwise over 5 min.. The resulting  
10 solution was stirred at -10 °C for 2.5 h. Water was then added and the mixture was concentrated under reduced pressure. The residue was poured in a saturated solution of NaHCO<sub>3</sub> and extracted with EtOAc. The organic phase was dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The product was purified by chromatography using silica gel (25% EtOAc/Hex) affording 530 mg  
15 (81% yield) of a pale yellow solid **10c**.

C. tBuOK (431 mg 2.1 eq.) was added to a suspension of the substrate **10c** (500 mg, 1.8 mmol) in 8 ml of tBuOH. The resulting mixture was then heated to 75°C for 7 h. The solution was allowed to reach room temperature overnight and 2.5 ml of HCl (4N/dioxane) was added. The mixture was concentrated under reduced  
20 pressure and the residue obtained was diluted with EtOAc. NaOH 1N was added until a pH of 7 was obtained. The organic phase was separated and dried (MgSO<sub>4</sub>), filtered, and concentrated under reduce pressure to afford 145 mg of **10d** (31% yield) as a pale beige solid. <sup>1</sup>H NMR (400 MHz, DMSO-d): δ 7.99 (d, J = 8.9 Hz, 1H), 7.49 (s, 1H), 7.37 (s, 1H), 7.18 (s, 1H), 6.92 (d, J = 8.9 Hz, 1H), 6.31 (s, 1H), 3.87 (s, 3H), 3.84 (s,  
25 3H).

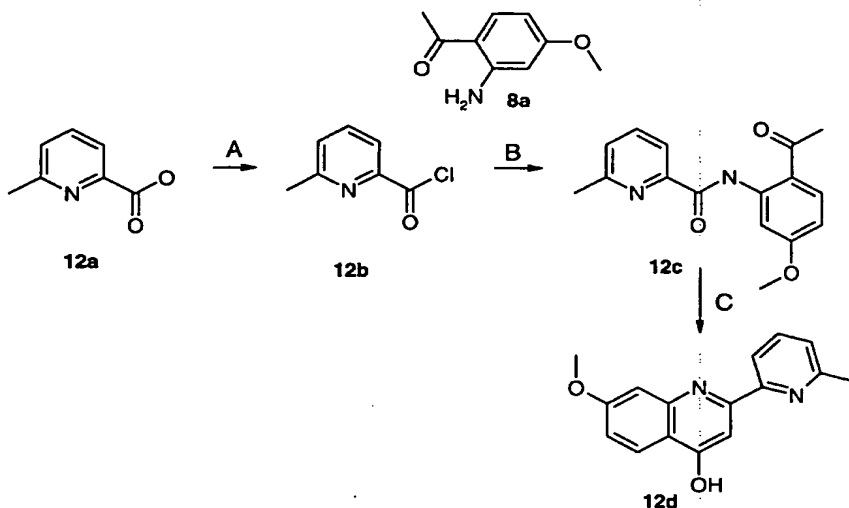
**EXAMPLE 11****Synthesis of 4-hydroxy-2(1-pyrrolyl)-7-methoxyquinoline (11b)**

- A. A solution of the substrate 11a (obtained from compound 6c after  
 5 hydrogenolysis of the benzyl group with 5% Pd/C in ethanol-THF) (1g, 5.25 mmol)  
 and 2,5-dimethoxytetrahydro furan (0.68 ml, 1 eq.) in glacial acetic acid was refluxed  
 for 4.5 h and allowed to reach RT. The mixture was then concentrated under  
 reduced pressure. The residue was diluted with methanol and NaOH(aq.) 1N was  
 added until pH 7 is reached. The product was purified by chromatography using  
 10 silica gel (3%MeOH/CH<sub>2</sub>Cl<sub>2</sub>, the residue was pre-adsorbed on silica gel). 140 mg  
 (13% yield) of 11b as a white solid was obtained.

<sup>1</sup>H NMR (400 MHz, DMSO-d): δ 7.98 (d, J = 9.2 Hz, 1H), 7.64 (s, 2H), 7.18 (d, J = 2.5  
 Hz, 1H), 7.05 (br. d, J = 7.9 Hz, 1H), 6.88 (br. s, 1H), 6.32 (s, 2H), 3.90 (s, 3H).

**EXAMPLE 12**

- 15 **Synthesis of 4-hydroxy-7-methoxy-2-(6-methyl-2-pyridyl)quinoline (12d)**



- A. 6-Methylpicolinic acid 12a (411 mg, 3.0 mmol) and SOCl<sub>2</sub> (0.520 mL, 7.2  
 mmol, 2.4 eq.) were refluxed in benzene (5 mL) for 2 h. The solvent and excess

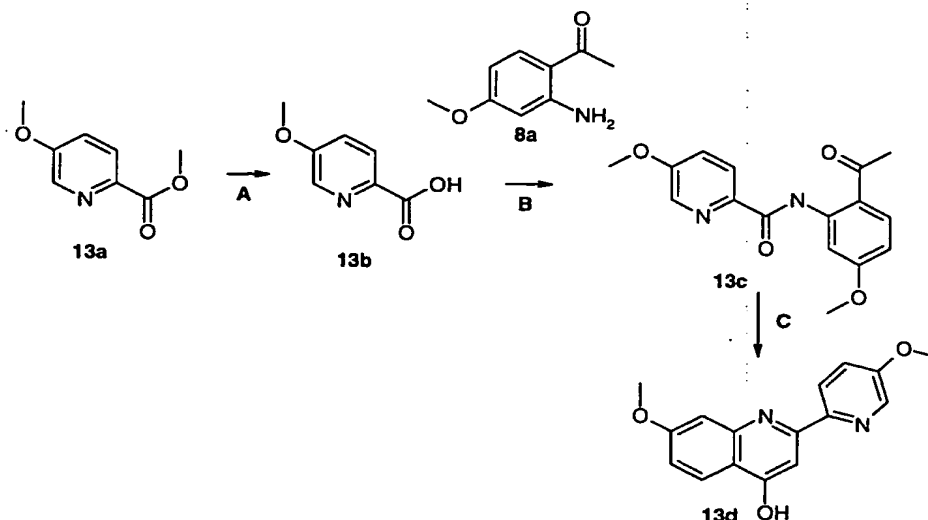
$\text{SOCl}_2$  were removed from the reaction mixture under vacuum and the residue was triturated with pentane. The solid material formed was filtered off and the filtrate concentrated to give the acid chloride **12b** (500 mg, 2.6 mmol).

B. To a solution of the crude acid chloride **12b** in  $\text{CH}_2\text{Cl}_2$  (5 mL) at  $0^\circ\text{C}$ , a solution of the aniline **8a** (344 mg, 2.08 mmol), DIPEA (1.45 mL, 8.35 mmol) and DMAP (61 mg, 0.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added. The reaction mixture was stirred at RT for 16 h. The volatile components were removed under vacuum, the residue was dissolved in EtOAc and the solution was washed with 5%  $\text{NaHCO}_3$  (2x),  $\text{H}_2\text{O}$  and brine. The organic layer was then dried over  $\text{MgSO}_4$  and concentrated under vacuum. The mixture was purified by flash column chromatography, using EtOAc/ hexane (1:2) as the eluent, to obtain the amide **12c** (490 mg, 82%).

C. To a suspension of amide **12c** (490 mg, 1.71 mmol) in t-BuOH (10 mL), tBuOK (410 mg, 3.43 mmol) was added and the mixture was stirred at  $75^\circ\text{C}$  for 6 h and then at RT for 16 h. The mixture was then poured in phosphate buffer (175 mL, pH= 7) and stirred for 30 min. The solid was triturated twice with ethyl acetate. The organic phase was washed with brine, dried over  $\text{MgSO}_4$  and concentrated under vacuum. The solid obtained was triturated with EtOAc to give the quinoline derivative **12d** (263 mg, 58%).  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  2.68 (s, 3 H), 3.94 (s, 3 H), 6.85–6.88 (2d,  $J$  = 8.68 & 9.5 Hz, 2 H), 6.94 (dd,  $J$  = 8.9 & 2.2 Hz, 1 H), 7.27 (dd,  $J$  = 6.7 & 1.9 Hz, 1 H), 7.73–7.79 (m, 2 H), 8.28 (d,  $J$  = 8.9 Hz, 1 H), 10.3 (br s, 1 H).

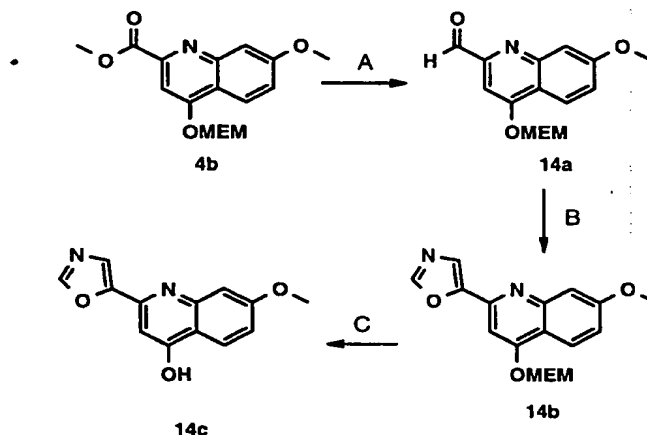
## EXAMPLE 13

## Synthesis of 4-hydroxy-7-methoxy-2-(5-methoxy-2-pyridyl)quinoline (13d)



- A. To a solution of compound 13a (623 mg, 3.73 mmol) in MeOH, NaOH (2M, 4.70 mL) was added and the reaction mixture was stirred at RT for 2 h. The solution was then acidified with HCl (6N, 2.2 mL) and concentrated to obtain compound 13b, which was used in the following step without purification.
- B. To a solution of the crude compound 13b (~3.73 mmol) in pyridine (25 mL), the aniline 8a (500 mg, 3.03 mmol) was added and the solution was cooled to -25 °C before POCl<sub>3</sub> (0.35 mL, 3.73 mmol) was added. The reaction mixture was stirred at -10 °C for 1 h and then at 0 °C for 2 h. The mixture was then poured onto H<sub>2</sub>O and extracted with EtOAc (2-3x). The combined organic layers were washed with 5% NaHCO<sub>3</sub> and brine, dried over MgSO<sub>4</sub> and concentrated under vacuum. The crude material was purified by flash column chromatography, using EtOAc/ hexane (1:2) as the eluent, to give the amide 13c (617 mg, 55%).
- C. To a suspension of the amide 13c (617 mg, 2.05 mmol) in anhydrous t-BuOH (10 mL), tBuOK (490 mg, 4.11 mmol) was added and the mixture was stirred at 75 °C for 6 h and then at RT for 16 h. The reaction mixture was poured in phosphate buffer (175 mL, pH= 7) and stirred for 30 min. The solid material formed was filtered and triturated with EtOAc to give the quinoline derivative 13d (250 mg,

43%).  $^1\text{H}$  NMR: (DMSO, 400 MHz):  $\delta$  3.86 (s, 3 H), 3.94 (s, 3 H), 6.72 (bs, 1 H), 6.91 (dd,  $J = 8.9$  & 1.9 Hz, 1 H), 7.54 (d,  $J = 1.9$  Hz, 1 H), 7.60 (dd,  $J = 8.9$  & 2.9 Hz, 1 H), 7.97 (d,  $J = 8.9$  Hz, 1 H), 8.21 (d,  $J = 8.6$  Hz, 1 H), 8.48 (d,  $J = 1.9$  Hz, 1 H).

**EXAMPLE 14****5 Synthesis of 4-hydroxy-7-methoxy-2-(oxazol-5-yl) quinoline (14c):**

- A. The protected quinoline derivative 4b from Example 4 (3.8 g, 11.8 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (60 mL) and cooled to  $-78^\circ\text{C}$  before diisobutylaluminum hydride (7.9 mL, 1 equiv., 1.5M in toluene) was added very slowly over 15 min.
- 10 After stirring for 80 min, an additional amount of DIBAL was added (5.5 mL, 0.7 equiv., 1.5 M in toluene). After stirring at  $-78^\circ\text{C}$  a further 2 h, the reaction was carefully quenched with methanol (4 mL) at  $-78^\circ\text{C}$  and then poured into aqueous solution of Rochelle salt (1N K-Na tartrate). The thick paste was stirred with  $\text{CH}_2\text{Cl}_2$  (300 mL) for 2 h until clear. The phases were separated and the organic phase dried
- 15 ( $\text{MgSO}_4$ ), filtered and concentrated to give a white solid. Purification by flash chromatography ( $\text{SiO}_2$ , 230-400 mesh) with 50% EtOAc /hexane gave aldehyde 14a as a white solid (2.5g, 73%).
- B. To a stirred suspension of  $\text{K}_2\text{CO}_3$  (48 mg, 0.34 mmol) in MeOH (7 mL) was added toluenesulphonylmethylisocyanide (66 mg, 0.34 mmol). The reaction was
- 20 heated to  $45^\circ\text{C}$  and aldehyde 14a (0.10 g, 0.34 mmol) was added. The reaction mixture was heated to  $80^\circ\text{C}$  for 16 h and then concentrated to dryness under

vacuum. Purification was performed by flash chromatography ( $\text{SiO}_2$ , 230-400 mesh) to afford the desired oxazole **14b** (0.089 g, 80%). MS: 331.0 ( $M + H$ )<sup>+</sup>.

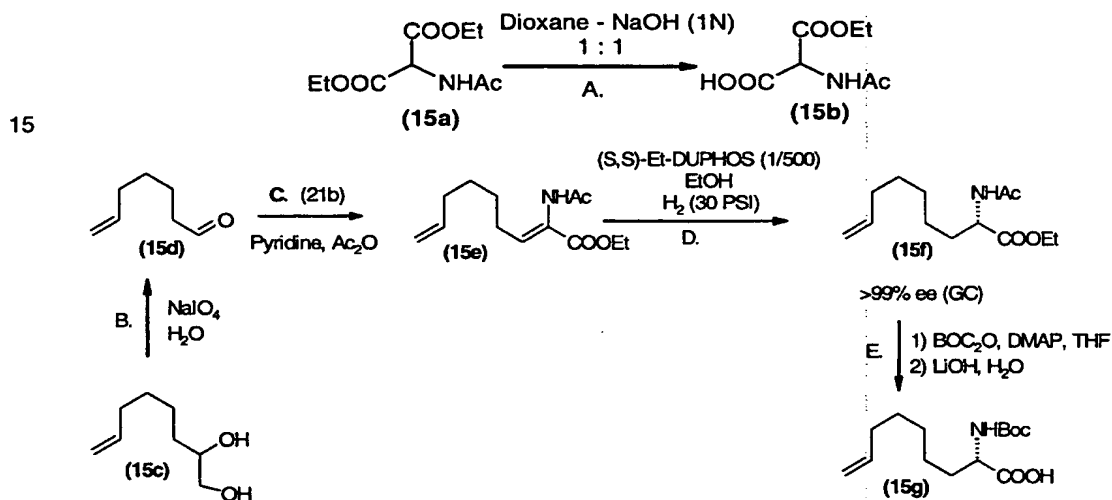
C. The MEM protected hydroxyquinoline **14b** was dissolved in THF (3 mL) and treated with aqueous HCl (1N, 1 mL). The reaction was stirred for 30 min at RT before being concentrated to dryness under vacuum. The residue was treated with phosphate buffer (3 mL, 1 N solution, pH 4.5) and stirred before the product was filtered out, washed with distilled water and dried overnight under high vacuum (60 °C, 16 h). The desired hydroxy quinoline **14c** was obtained as a tan colored solid (0.065 g, 100%). MS: 242.9 ( $M + H$ )<sup>+</sup>.

<sup>1</sup>H NMR ( $\text{DMSO}-d_6$ ):  $\delta$  8.65 (s, 1H), 8.02 (bs, 1H), 7.97 (d,  $J = 8.9$  Hz, 1H), 7.19 (s, 1H), 6.93 (d,  $J = 7.9$  Hz, 1H), 6.42 (bs, 1H), 3.87 (s, 3H). ES (+) MS:  $m/z$  242.9 ( $M + H$ )<sup>+</sup>.

#### PEPTIDE LINKER MOIETIES (P3)

##### EXAMPLE 15

##### Synthesis of (2S)-N-Boc-amino-non-8-enoic acid (**15g**)



A. To a solution of commercially available diethyl 2-acetamidomalonate **15a** (100 g, 0.46 mole) in dioxane (500 mL) was added aqueous sodium hydroxide (1 M, 1 eq., 460 mL) dropwise over 30 to 45 min. The resulting mixture was left to stir for 16.5 h, then dioxane was evaporated *in vacuo*, the aqueous solution was extracted with three portions of 300 mL of ethyl acetate and acidified to pH 1 with

concentrated HCl. This solution was left to crystallize in an ice-water bath. After the appearance of a few crystals, the mixture was sonicated and an abundant precipitate appeared. Filtration and drying under vacuum afforded compound 15b, (62.52 g, 72% yield) as a white solid.

5 B. To a magnetically stirred emulsion of commercially available 7-octene-1,2-diol 15c (25 g, 0.173 mole) and H<sub>2</sub>O (100 mL), in a 1 L round bottom flask, an aqueous solution of sodium periodate (40.7 g, 0.190 moles, 1.1 eq., in 475 mL H<sub>2</sub>O) was added over a period of 20 min (slightly exothermic). The resulting mixture was stirred at room temperature for an additional 1 h (completion of reaction confirmed  
10 by TLC). The mixture was then decanted in a separatory funnel and the aqueous layer was separated from the organic layer. The aqueous solution was saturated with NaCl, decanted and separated from the organic fraction once more. The two organic fractions were combined, dried with sodium sulfate and filtered over a cotton plug (in a Pasteur pipette) to give compound 15d (15.135 g, colorless oil, 78%  
15 yield). The aqueous solution was extracted with CH<sub>2</sub>Cl<sub>2</sub>, dried with anhydrous MgSO<sub>4</sub>, and concentrated under vacuum (without heating, heptanal b.p.153°C) to obtain an additional amount of compound 15d (1.957 g, colorless oil, 10% yield). Total yield 88%.

C. To solid ethyl 2-acetamidomalonate 15b (7.57 g, 40 mmol.) was added 6-  
20 heptenal 15d (4.48 g, 40 mmol) in solution in pyridine (32 mL, 10 eq) over 1 min. The resulting solution was cooled in a 10° C bath and acetic anhydride (12 mL, 3.2 eq.) was added over 4 min. The resulting orange solution was stirred for 3 h at RT and another portion of ethyl 2-acetamidomalonate 15b (2.27 g) was added. The resulting mixture was stirred at room temperature for an extra 11 h. Ice (60 mL) was  
25 then added and the solution was stirred for 1.5 h, then the mixture was diluted with 250 mL of water and extracted with two portions of ether. The ethereal solution was washed with 1N HCl, sat. NaHCO<sub>3</sub>, dried Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (EtOAc 40% / hexane) to give compound 15e (4.8g, 50% yield) as a pale yellow oil.

30 D. To a degassed (argon bubbling for 30 min.) solution of Z-ethyl 2-acetamido-2,8-nonadienoate 15e (8.38g, 35 mmol) in dry ethanol (70 mL) was added (S,S)-Et-

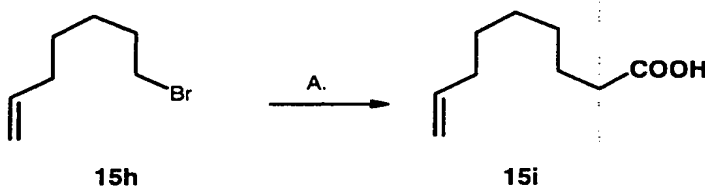


DUPHOS Rh(COD)OTf (51 mg, S/C = 496). The mixture was put under 30 psi of hydrogen (after 4 vacuum-H<sub>2</sub> cycles) and stirred on a Parr shaker for 2 h. The resulting mixture was evaporated to dryness to obtain the crude compound 15f, which was used in the subsequent step without purification.

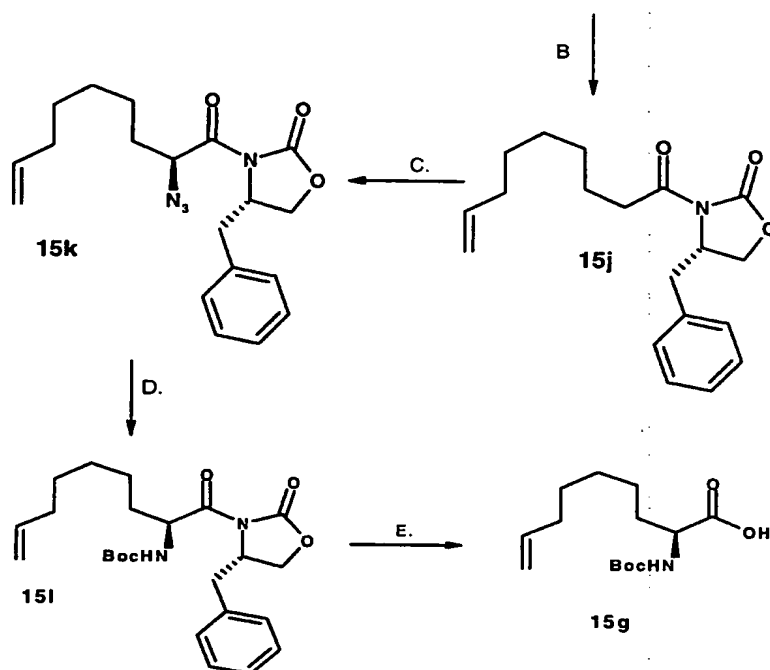
- 5 E. To a solution of crude (S)-ethyl 2-acetamido-8-nonenoate 15f (7.3 g, 30.3 mmol) in THF (100 mL), Boc<sub>2</sub>O (13.2 g, 2 eq.) and DMAP (740 mg, 0.2 eq) were added, and the reaction mixture was heated at reflux for 2.5 h. Subsequently, most of the THF solvent was evaporated, the crude mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with 1 N HCl in order to remove the DMAP. The organic layer was further
- 10 extracted with saturated aqueous NaHCO<sub>3</sub>, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was then diluted with THF (50 mL) and water (30 mL), LiOH.H<sub>2</sub>O (2.54 g, 2 eq.) was added and the resulting mixture was stirred at RT for 25 h (completion of the hydrolysis was confirmed by TLC). The reaction mixture was concentrated under vacuum to remove most of the THF
- 15 solvent and diluted with CH<sub>2</sub>Cl<sub>2</sub>. The resulting solution was washed with 1 N HCl, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. In order to remove minor impurities and excess Boc<sub>2</sub>O, the crude product was purified by flash chromatography (using a solvent gradient from 100% hexane – 100% EtOAc as the eluent). The titled compound 15g was obtained in high purity as a pale yellow oil
- 20 (5.82 g, 71% yield). <sup>1</sup>H NMR (DMSO, 400 MHz): δ 7.01 (d, J = 8 Hz, 1H), 5.79 (tdd, J<sub>t</sub> = 6.7 Hz, J<sub>d</sub> = 17.0, 10.2 Hz, 1H), 5.00 (md, J<sub>d</sub> = 17.0 Hz, 1H), 4.93 (md, J<sub>d</sub> = 10.2 Hz, 1H), 3.83 (m, 1H), 2.00 (q, J = 6.9 Hz, 2H), 1.65-1.5 (m, 2H), 1.38 (s, 9H), 1.35-1.21 (m, 6H).

#### EXAMPLE 15A

- 25 Alternative synthesis of (2S)-N-Boc-amino non-8-enoic acid (15g):



56



- A. To a stirred suspension of finely cut Mg ribbons (0.55 g, 22.5 mmol) in dry THF (30 mL) containing dibromoethane (0.1 mL), 8-bromo-1-octene (15h, 2.52 mL, 15 mmol) was added dropwise over a period of 15 min, [the reaction is slightly exothermic]. After 30 min, the mixture was heated to 38° for 1 h and then cooled to -78° before it was added via a cannula onto an excess amount of solid CO<sub>2</sub>. The mixture was diluted with diethyl ether (100 mL) and the solution was washed with brine (2 x 50 mL), dried over MgSO<sub>4</sub> and evaporated. A crude oil was obtained which was purified by chromatography on silica gel using 15% EtOAc in hexanes as the eluent to give compound 15i in 62% yield (1.44 g).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.31-1.42 (m, 6H), 1.60-1.69 (m, 2H), 2.02-2.09 (m, 2H), 2.35 (t, J = 8.3 Hz, 2H), 4.99 (dm, J = 10.0 Hz, 1H), 5.04 (dm, J = 17.0 Hz, 1H), 5.75-5.86 (m, 1H).

- B. To a vigorously stirring solution of the carboxylic acid 15i (1.36g, 8.7 mmol) in anhydrous THF (70 mL) at -78°, freshly distilled Et<sub>3</sub>N (1.6 mL; 11.3 mmol) and pivaloyl chloride (1.18 mL, 9.58 mmol) were added via a syringe under anhydrous conditions. The mixture was stirred at -78° for 15 min and then at 0° for 45 min.

The mixture was cooled again to  $-78^{\circ}$  and then transferred via a cannula into an anhydrous solution of 4(S)-4-(phenylmethyl)-2-oxazolidinone lithium salt in THF at  $-78^{\circ}$ ; the lithium salt of the oxazolidinone reagent had been previously prepared by the slow addition of *n*-BuLi (2.00 M in hexanes, 7.85 mL, 15.7 mmol) into a THF (20 mL) solution of the oxazolidinone (2.78g, 15.7 mmol) in THF at  $-78^{\circ}$ .

The reaction mixture was stirred at  $-78^{\circ}$  for 15 min then at RT for 1.5 h. Finally, it was quenched with an aqueous solution of sodium bisulfate (100 mL of 1 M) and the THF evaporated to  $\frac{3}{4}$  of its initial volume. The residue was extracted with EtOAc (2 x 150 mL) and the mixed organic layers were washed with 5%  $\text{NaHCO}_3$  (3 x 50 mL), brined (2 x 50 mL), dried over  $\text{MgSO}_4$  and evaporated. The resulting crude oil was chromatographed on silica gel, using 15% EtOAc in Hexanes to obtain compound 15j in 68% yield (1.88g).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.35-1.47 (m, 6H), 1.67-1.74 (m, 2H), 2.02-2.09 (m, 2H), 2.65 (dd,  $J = 13.4$  & 9.9 Hz, 1H), 2.84-3.02 (m, 2H), 3.31 (dd,  $J = 13.4$  & 3.2 Hz, 1H), 4.13-4.22 (m, 2H), 4.62-4.71 (m, 1H), 4.93 (d,  $J = 10.2$  Hz, 1H), 5.00 (dd,  $J = 17.2$  & 1.6 Hz, 1H), 5.75-5.84 (m, 1H), 7.18-7.38 (m, 5H).

C. To a stirred solution of KHMDS (0.8 M THF, 22 mL, 17.5 mmol) in dry THF (50 mL) at  $-78^{\circ}$  was cannulated a solution of the acid derivative 15j (3.25g, 10.30 mmol) in dry THF (40 mL) at  $-78^{\circ}$ . The mixture was stirred at  $-78^{\circ}$  for 45 min. To this mixture, a solution of trizylazide (3.67g, 11.85 mmol) in dry THF (40 mL) at  $-78^{\circ}$  was added. The mixture was stirred at  $-78^{\circ}$  for 3 min then quenched with acetic acid (5 mL). Subsequently, it was stirred at RT for 1 h and 45 min and finally at  $40^{\circ}$  for 15 min. Most of the THF was evaporated. The residue was taken into EtOAc (100 mL) and the organic solution washed with  $\text{H}_2\text{O}$  (50 mL), 5%  $\text{NaHCO}_3$  (3x 50 mL) and brine (50 mL), ( $\text{MgSO}_4$ ) and evaporated. The oil obtained was chromatographed on silica gel using Hexane/ $\text{CH}_2\text{Cl}_2$  (1/1) as the eluent to give compound 15k (2.47g, yield 67%).

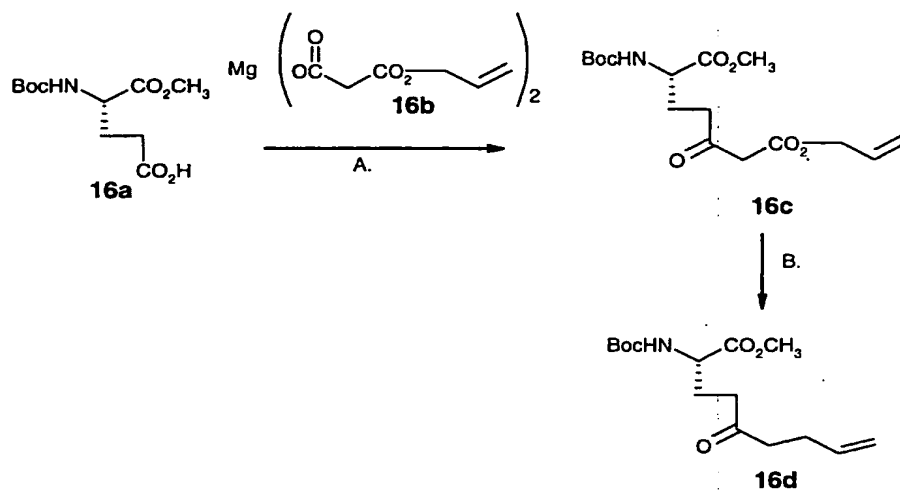
$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.32-1.45 (m, 6H), 1.45-1.6 (m, 1H), 1.75-1.88 (2, 2H, rotamers), 2.01-2.11 (m, 2H), 2.82-2.87 (m, 1H), 3.33 (dd,  $J = 13.4$  & 3.2 Hz, 1H), 4.10-4.28 (m, 2H), 4.62-4.72 (m, 1H), 4.90-5.05 (m, 3H), 5.73-5.88 (m, 1H), 7.17-7.38 (m,

5H).

D. To a stirred solution of anhydrous  $\text{SnCl}_2$  (2.61 g, 13.8 mmol) in anhydrous MeOH (80 mL), a solution of the azide **15k** (2.45 g, 6.9 mmol) was cannulated at 0° in anhydrous MeOH (20 mL). The mixture was stirred at RT for 4 h. The MeOH was  
5 evaporated and the foamy material obtained was taken into dioxane/ $\text{H}_2\text{O}$  (100  $\mu\text{L}$ /20  $\mu\text{L}$ ) and treated with  $\text{Boc}_2\text{O}$  (3.0 g, 13.8 mmol) and  $\text{NaHCO}_3$  (2.89 g, 34.5 mmol) (pH adjusted to 8 with more  $\text{NaHCO}_3$  if needed) and the mixture was stirred at RT for 16 h. Part of the dioxane was evaporated (~50%) and the residue was extracted twice with EtOAc. The organic solution was washed with brine (2 x 50  
10 mL), dried and evaporated. The residue obtained was chromatographed on silica gel using 20-25% EtOAc in hexane as eluent to give the compound **15l** (1.75 g, yield 60%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.27-1.53 (m, 6H), 1.46 (s, 9H), 1.80 (m, 1H), 2.00-2.08 (m, 1H), 2.80 (t,  $J = 12.1$  Hz, 1H), 3.34 (d, 14.3 Hz, 1H), 4.17-4.23 (m, 2H), 4.60-4.66  
15 (m, 1H), 4.93 (d,  $J = 10.2$  Hz, 1H), 5.05 (dd,  $J = 17.2$  & 1.9 Hz, 1H), 5.13 (bs, 1H), 5.38-5.43 (m, 1H), 5.74-5.84 (m, 1H), 7.22-7.36 (m, 5H).

E. To a stirred solution at 90° of the N-Boc derivative **15l** (1.74 g, 4.04 mmol) in THF/ $\text{H}_2\text{O}$  (75 mL/15 mL),  $\text{H}_2\text{O}_2$  (30% v/w, 2.05 mL, 16.2 mmol) and  $\text{LiOH}\cdot\text{H}_2\text{O}$  (0.34 g, 8.1 mmol) were added and the solution was stirred at 0° for 1 h. The  
20 reaction was quenched with  $\text{Na}_2\text{SO}_3$  (2.24 g in  $\text{H}_2\text{O}$ , 15 mL, 17.8 mmol). The pH was adjusted to 4-5 with 10% aqueous citric acid and the mixture diluted with EtOAc. The aqueous fraction was extracted once more with EtOAc and the organic solution was washed twice with brine, dried and evaporated. The residue was chromatographed on silica gel using 20% hexane in EtOAc as the eluent to give the  
25 free carboxylic acid **15g** (0.76 g, yield 70%). This compound was identical in all respects to the one obtained in example 15.

**EXAMPLE 16****Synthesis of (2S)-N-Boc-amino-5-oxo-non-8-enoic acid methyl ester (16d):**

This synthesis is based on methodology by T. Tsuda et al., *J. Am. Chem. Soc.*, 1980,  
 5 102, 6381-6384.

A. To a well stirred solution of the monoallyl ester of malonic acid (1.50 g, 10.4 mmol) in dry THF under N<sub>2</sub> (20 mL) at -78° n-Bu<sub>2</sub>Mg (0.9M/hexane, 5.8 mL, 5.2 mmol) was added dropwise over a period of 5 min. The heavy suspension was then stirred at RT for 1 h and evaporated to dryness (vacuum release under N<sub>2</sub>). The  
 10 solid Mg salt 16b, was dried under vacuum for 1 h.

Glutamic acid derivative 16a was first mixed with 1,1'-carbonyldiimidazole (1.65 g, 10.21 mmol) in anhydrous THF and the mixture was stirred at RT for 1 h in order to activate the free acid moiety. Subsequently, the activated glutamic acid derivative was cannulated into a solution of the Mg salt 16b and the reaction mixture obtained  
 15 was stirred at RT for 16 h. It was then diluted with EtOAc and the organic solution was washed with 0.5 N ice-cold HCl, brined, dried and evaporated. The residue obtained was chromatographed on silica gel using 35-40% EtOAc in hexane as eluent to give compound 16c (1.85 g, yield 53%).

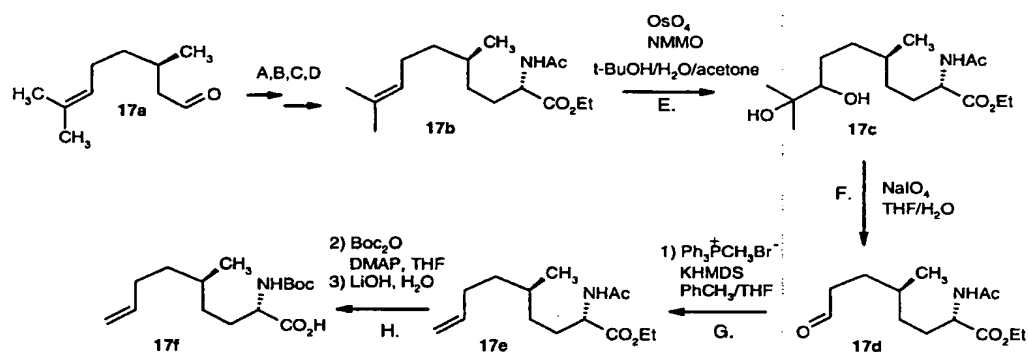
<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.44 (s, 9H), 1.85-1.95 (m, 1H), 2.12-2.22 (m, 1H), 2.58-  
 20 2.74 (m, 2H), 3.48 (s, 2H), 3.74 (s, 3H), 4.24-4.34 (m, 1H), 4.52 (dm, J = 5.7 Hz, 2H), 5.09 (m, 1H), 5.25 (dm, J = 10.2 Hz, 1H), 5.34 (dm, J = 17.2 Hz, 1H), 5.91 (m, 1H).

B. To a stirred solution of tetrakis (triphenylphosphine) Pd (0) (0.116 g, 5 mol %, 0.1 mmole) in dry DMF (7 mL) was added (via a siring and under a N<sub>2</sub> atmosphere) the diester 16c (0.687 g, 2 mmol) in dry DMF (3 mL). The mixture was stirred at RT for 3.5 h. The DMF was evaporated under reduced pressure and the residue diluted with EtOAc (20 mL). The EtOAc solution was washed with 0.5 N ice-cold HCl (5 mL), brine (10 mL), dried and evaporated. The residue was chromatographed on silica gel using 15-20% EtOAc in hexane as eluent to give compound 16d (0.253 g, yield 42%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.44 (s, 9H), 1.84-1.94 (m, 1H), 2.08-2.22 (m, 1H), 2.33 (dd, J = 14.0 & 7.3 Hz, 2H), 2.45-2.55 (m, 4H), 3.74 (s, 3H), 4.28 (bm, 1H), 4.98 (dm, J = 10.2 Hz, 1H), 5.03 (dm, J = 17.2 Hz, 1H), 5.00-5.10 (m, 1H), 5.74-5.85 (m, 1H).

#### EXAMPLE 17

##### Synthesis of (2S,5R)-N-Boc-2-amino-5-methyl-non-8-enoic acid (17f)



A,B,C,D. Commercially available (R)-(+)-citronellal 17a was first converted to the amino acid derivative 17b following the same synthetic steps as those previously described in Example 15 for the conversion of aldehyde 15d to amino acid intermediate 15f.

E. Compound 17b (0.675 g, 5.6 mmol) was dissolved in a mixture of tBuOH/acetone/H<sub>2</sub>O (1:1:1, 18 mL) and placed in an ice bath (0°C). NMMO (0.789 g, 6.74 mmol, 1.2 eq.) and OsO<sub>4</sub> (2.5% w/w in tBuOH, 0.7 mL, 0.067 mmol., 0.012 eq) were added consecutively and the reaction mixture was stirred at RT for 4 h. Most of the acetone was removed by evaporation under vacuum and then the mixture

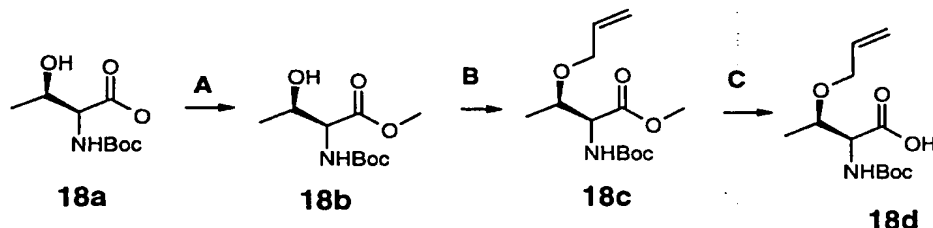
was extracted with EtOAc. The organic layer was further washed with H<sub>2</sub>O and brine, dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness. The diol 17c was obtained in high purity after flash column chromatography using 1% EtOH in EtOAc as the eluent in 77% yield (0.575 g).

- 5 F. To a solution of diol 17c (0.575 g, 1.73 mmol) in THF/H<sub>2</sub>O (1:1, 20 mL) at 0°C, NaIO<sub>4</sub> (0.48 g, 2.25 mmol, 1.3 eq.) was added and the reaction mixture was stirred at RT for 3.5 h. Most of the THF solvent was subsequently removed by evaporation under vacuum and the remaining mixture was extracted with EtOAc (2x100mL). The combined organic layers were further washed with 5% aqueous citric acid  
10 solution (2x20 mL), 5% aqueous NaHCO<sub>3</sub> (20 mL) and brine (2x50 mL), then the EtOAc solution was dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness under vacuum. The aldehyde intermediate 17d (0.47 g of crude product) was used in the next step without further purification.

- G. To a solution of Ph<sub>3</sub>PCH<sub>3</sub>Br (925 mg, 2.6 mmol) in anhydrous toluene (15  
15 mL), KHMDS (0.5M in toluene, 5.2 mL, 2.6 mmol) was added and the yellow suspension formed was stirred at RT for 30 min under N<sub>2</sub>. After that period, the suspension was first cooled to 0°C, a solution of the aldehyde 17d (0.47 g 1.73 mmol, dissolved in 15 mL of anhydrous THF) was added *via* a syringe and the mixture was allowed to warm-up to RT. After stirring at RT for 1 h, most of the THF was  
20 removed by evaporation under vacuum, EtOAc (100 mL) was added to the mixture and the organic layer was washed with H<sub>2</sub>O (30 mL), 5% aqueous NaHCO<sub>3</sub> (30 mL) and brine (30 mL). The EtOAc solution was then dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness under vacuum. Pure compound 17e was isolated after purification by flash column chromatography on silica gel, using hexane:EtOAc (3:2)  
25 as the eluent, in 63% yield (0.29 g) for the two last steps.

The hydrolysis of the ethyl ester and simultaneous exchange of the *N*-acetyl protecting group for a Boc in intermediate 17e to obtain compound 17f was carried out using the same procedure as that reported for the conversion of compound 15f to 15g (17f, 310 mg, quantitative). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.88 (d, J=6.4 Hz,

3H), 1.18-1.28 (m, 2H), 1.35-1.48 (m, 3H), 1.45 (s, 9H), 1.64-1.74 (m, 1H), 1.81-1.89 (m, 1H), 1.94-2.12 (m, 2H), 4.28 (bd,  $J = -3.2$  Hz, 1H), 4.93 (dm,  $J = 11.1$  Hz, 1H), 5.00 (dm,  $J = 16.8$  Hz, 1H), 5.74-5.84 (m, 1H).

**EXAMPLE 18****5 Synthesis of *N*-Boc-*O*-allyl-(L)-threonine (18d)**

A. Boc-(L)-threonine 18a (500 mg, 2.28 mmol) was partially dissolved in  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (8 mL/0.5 mL, respectively) at  $0^\circ\text{C}$ . A solution of diazomethane in diethyl ether was slowly added until the yellow color persisted, indicating the presence of excess diazomethane. Upon evaporation of the solvents, crude methyl ester 18b was obtained as a cloudy white oil (0.534 g).

B. Intermediate 18b (311 mg, 1.33 mmol) was then dissolved in anhydrous diethyl ether (8 mL),  $\text{Ag}_2\text{O}$  was added (341 mg, 1.47 mmol) and freshly activated 4Å molecular sieves (1 g). Finally, allyl iodide (134  $\mu\text{L}$ , 1.47 mmol) was added to the reaction flask and the mixture was stirred at reflux. Two more portions of allyl iodide (45  $\mu\text{L}$ , 0.50 mmol, each time) were added after a period of 20 h and 30 h, and stirring was continued for a total of 36 hours. Then the mixture was filtered through celite and purified by flash column chromatography on silica gel, using  $\text{EtOAc}/\text{hexane}$  (1:4) as the eluent, to give 73 mg (27% yield) of compound 18c as a clear oil.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400MHz):  $\delta$  1.21 (d,  $J = 6.0$  Hz, 3H), 1.45 (s, 9H), 3.75 (s, 3H), 3.82-3.87 (m, 1H), 3.99-4.07 (m, 2H), 4.29 (dd,  $J = 9.5$  & 2.5 Hz, 1H), 5.14 (dm,  $J = 10.5$  Hz, 1H), 5.21 (dm,  $J = 17.2$  Hz, 1H), 5.75-5.84 (m, 1H).

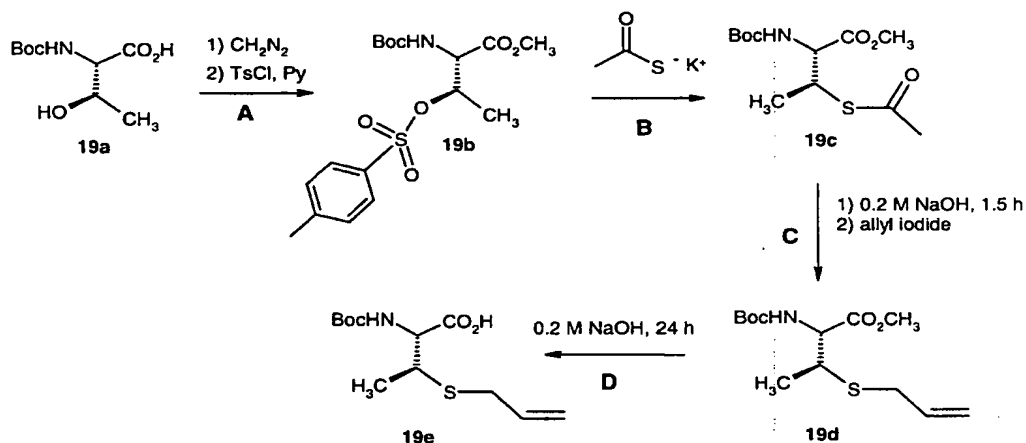
C. The ester compound 18c (99 mg, 0.362 mmol) was dissolved in a mixture of THF/MeOH/ $\text{H}_2\text{O}$  (2:1:1, 4 mL) and  $\text{LiOH}\cdot\text{H}_2\text{O}$  (61 mg, 1.45 mmol) was added. The solution was stirred at RT for 2 h, and was then acidified with 1N HCl to pH ~3



before the solvents were removed under vacuum. The resulting oil, compound 18d was used as such for the synthesis of macrocyclic inhibitors.

#### EXAMPLE 19

##### Synthesis of (2S, 3S)-N-Boc-2-amino-3(mercaptoallyl)butanoic acid (19e)



5

- A. Compound 19a (9.1 mmol) was dissolved in pyridine (5 mL) and the solution was cooled to 0°C in an ice bath, tosyl chloride (2.3 g, 11.8 mmol, 1.3 eq.) was added in small portions and the reaction mixture was stirred at RT for 24 h. After that period, the reaction mixture was partitioned between diethyl ether (300 mL) and H<sub>2</sub>O (100 mL). The ether layer was further washed with 0.2N HCl (6x100 mL) and brine (100 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated to dryness under vacuum. Purification of the crude material by flash column chromatography, using hexane/EtOAc (gradient from 8:2 to 7:3 ratio) as the eluent, led to the isolation of tosyl derivative 19b in 85% yield (3.05 g).
- 15 B. To solution of intermediate 19b (775 mg, 2 mmol) in anhydrous DMF (2.5 mL), potassium thioacetate (365 mg, 3.2 mmol, 1.6 eq.) was added and the reaction mixture was stirred at RT for 24 h. Most of the DMF was then evaporated under vacuum and the remaining mixture was partitioned between EtOAc and H<sub>2</sub>O. The aqueous layer was re-extracted with EtOAc, the combined organic layers were washed with brine, dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness.
- 20 Purification of the crude material by flash column chromatography using hexane/EtOAc (4:1 ratio) as the eluent, led to the isolation of compound 19c in 80%

yield (465 mg).

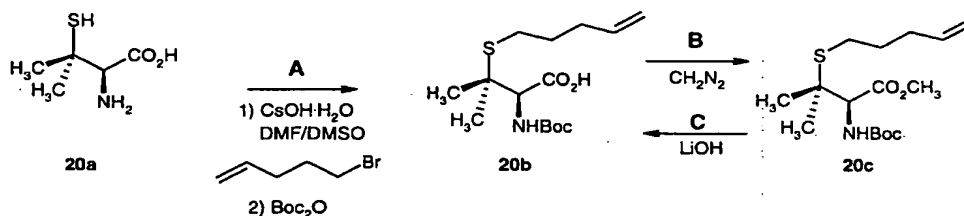
C. To a solution of thioester **19c** (465 mg) in H<sub>2</sub>O/EtOH (3:5 ratio, 8 mL), an aqueous solution of 0.2M NaOH (2.4 mL) was added and the mixture was stirred at RT for 1.5 h. Allyl iodide (0.292 mL, 3.2 mmol, 2 eq.) was then added and stirring  
5 was continued at RT for an additional 30 min. The reaction mixture was concentrated to half of its original volume and then extracted with EtOAc. The aqueous layer was acidified to pH=3 with cold aqueous 0.5N HCl and re-extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness under vacuum. The crude reaction  
10 mixture contained at least four products; all of the products were isolated after flash column chromatography on silica gel, using hexane/EtOAc (gradient from 9:1 to 3:1 ratio). The structure of the least polar compound (TLC R<sub>f</sub> = 0.68 in hex/EtOAc 4:1) corresponded to the desired product **19d** (83 mg, 18% yield).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 1.24 (d, J=7.0 Hz, 3H), 1.46 (s, 9H), 3.13-3.19 (m, 2H),  
15 3.24-3.29 (m, 1H), 3.77 (s, 3H), 4.50 (dd, J=8.6 & 3.8 Hz, 1H), 5.12 (d, J=12.4 Hz, 1H), 5.15 (dd, J=18.4 & 1.3 Hz, 1H), 5.22 (bd, J=7.6 Hz, 1H), 5.75-5.85 (m, 1H).

D. A solution of the methyl ester **19d** (83 mg, 0.287 mmol) in MeOH/H<sub>2</sub>O (3:1, 4 mL) was mixed with aqueous NaOH (0.2 N, 1.3 mL, 0.26 mmol) for 24 h at RT and for 1 h at 40 °C. The reaction mixture was acidified with cold aqueous HCl (0.5 N  
20 HCl, at 0 °C, pH=4-5), the MeOH was removed under vacuum and the remaining aqueous mixture was extracted with EtOAc. The organic solution was dried over MgSO<sub>4</sub> and evaporated to dryness in order to obtain compound **19e**. Compound **19e** was used for the final synthesis of inhibitors without any further purification.

## EXAMPLE 20

## Synthesis of (S)-N-Boc-2-amino-3-methyl-3(1-mercapto-4-butenyl)butanoic acid (20c)



5 A. L-Penicillamine 20a (448 mg, 3 mmol) was dissolved in DMF/DMSO (5:1 ratio, 6 mL), 4-bromopentene (0.46 mL, 4.5 mmol, 1.5 eq.) and  $\text{CsOH}\cdot\text{H}_2\text{O}$  (1.0g, 6mL, 2 eq.) were added and the reaction mixture was stirred at RT. After 24 h,  $\text{Boc}_2\text{O}$  (820 mg, 3.75 mmol, 1.25 eq.) was added to the mixture and stirring was continued for an additional 12 h. The DMF was subsequently removed under vacuum, the remaining  
 10 mixture was diluted with cold aqueous 0.5N HCl adjusting the pH= $\sim$ 4-5 and then extracted with EtOAc (2x50 mL). The organic layer was washed with brine (2x), dried over anhydrous  $\text{MgSO}_4$  and evaporated to dryness to give the crude carboxylic acid 20b.

B. Purification of 20b turned out to be difficult, thus the crude product was first  
 15 treated with diazomethane to form the corresponding methyl ester 20c, and then purified by flash column chromatography, using hexane/EtOAc (9:1) as the eluent, to obtain 190 mg (20% yield) of the pure methyl ester 20c.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.35 (s, 3H), 1.37 (s, 3H), 1.44 (s, 9H), 1.59-1.67 (m, 2H), 2.11-2.17 (m, 2H), 2.51-2.60 (m, 2H), 3.74 (s, 3H), 4.29 (d,  $J$ = 8.6 Hz, 1H), 4.98 (dm,  $J$ =10.5 Hz, 1H), 5.03 (dm,  $J$ =19 Hz, 1H), 5.35 (bd,  $J$ =7 Hz, 1H), 5.72-5.83 (m, 1H).  
 20

C. The ester was subsequently dissolved in THF/MeOH/ $\text{H}_2\text{O}$  (2:2:1, 5mL),  $\text{LiOH}\cdot\text{H}_2\text{O}$  (50 mg, 2.0 mmol, 2 eq.) was added and the reaction mixture stirred at  $40^\circ\text{C}$  for 4 h to hydrolyze the ester 20c back to the acid 20b. The reaction mixture was acidified with 0.5N HCl to pH=4-5, the THF and MeOH were evaporated to  
 25 dryness and the remaining aqueous solution was extracted with EtOAc. The EtOAc

layer was dried over anhydrous  $\text{MgSO}_4$ , and evaporated to dryness to give compound **20b**, which was used in the subsequent synthesis of macrocyclic inhibitors without further purification.

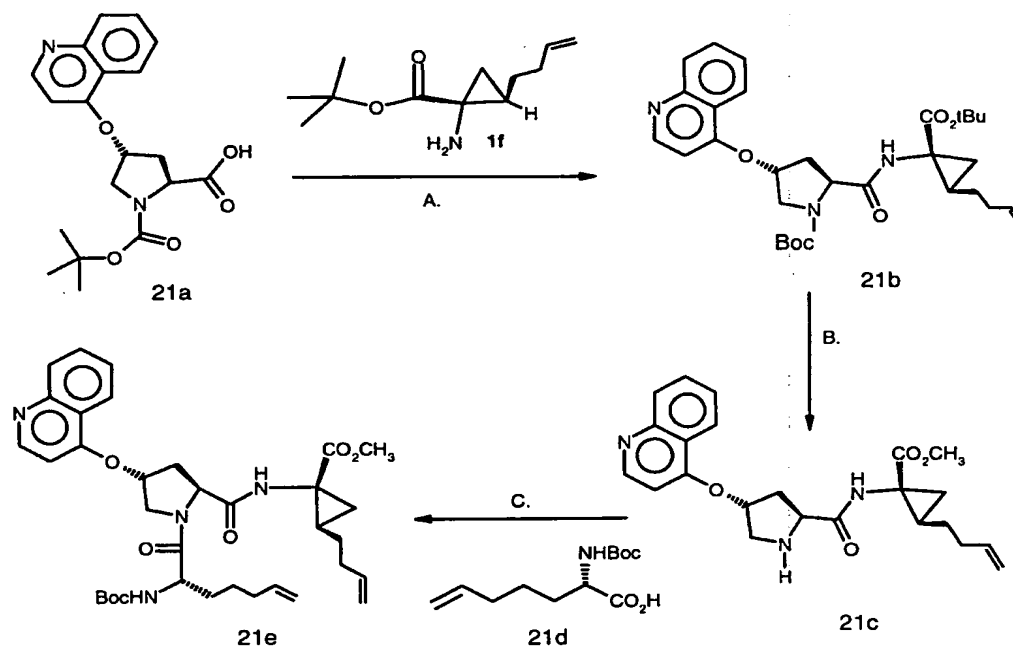
## 5 ACYCLIC DIPEPTIDE AND TRIPEPTIDE INTERMEDIATES

The general procedure for coupling reactions done in solution and specific examples thereof are described in WO 00/09543 and WO 00/09558.

These procedures have been used for the synthesis of the intermediate dipeptides **26c**, **30a** and tripeptides **23a**, **24a**, **31a**, **32a**, and **33a**.

## 10 EXAMPLE 21

### Synthesis of acyclic tripeptide **21e**



- A. To a solution of the proline derivative **21a** (prepared from commercially available Boc-4(R)-hydroxyproline and 4-chloro-quinoline as described in WO 00/05543 and WO 00/09558) (1.32 g, 3.68 mmol) and the crude homoallyl ACCA **1f** (~3.35 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL), NMM (1.21 mL, 10.05 mmol) and HATU (1.53, 4.02 mmol) were added in succession and the suspension was stirred at RT for 18 h. After that period, the solvent was evaporated and the crude reaction mixture was

redissolved in EtOAc (30 mL). The solution was washed with 5% aqueous  $\text{NaHCO}_3$  (2 x 10 mL), brine (10 mL), dried over  $\text{MgSO}_4$  and evaporated. The crude product was purified by chromatography on silica gel using 8% diethyl ether in EtOAc as the eluent to obtain the desired diastereomer of compound 21b in 20% yield (the absolute stereochemistry was not determined).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.93 & 1.01 (t,  $J$  = 8.3 Hz, 1H, rotamers in ratio of 3:7), 1.14-1.35 (m, 2H), 1.44 (s, 9H), 1.45 (s, 9H), 1.50-1.82 (m, 4H), 2.08-2.24 (m, 2H), 2.32 (bs, 0.7H), 2.63 (bs, 0.75H), 2.93 (bs, 0.75H), 3.16 (m, 0.25H), 3.77 (bs, 1.5H), 3.88 (bs, 0.5H), 4.4-4.55 (m, 1H), 4.98 (d,  $J$  = 10.2 Hz, 1H), 5.03 (dd,  $J$  = 17.2 & 1.6 Hz, 1H), 5.24 (bs, 1H), 5.75-5.88 (m, 1H), 6.57 & 6.78 (2bs, 1H, 2 rotamers), 7.42-7.58 (m, 3H), 7.63-7.73 (m, 2H), 8.04 (d,  $J$  = 8.3 Hz, 1H), 8.11 (d,  $J$  = 8.3 Hz, 1H), 8.74 (d,  $J$  = 5.1 Hz, 1H).

B. To a solution of the dipeptide 21b (137 mg, 0.248 mmol) in dry  $\text{CH}_2\text{Cl}_2$ , a solution of HCl in dioxane (4M, 4 mL) was added and the mixture was stirred at RT for 1.5 h. The solvent was then evaporated and the residue dried under high vacuum to give the free amino acid. The mixture was dissolved in diethyl ether/MeOH (3  $\mu\text{L}$ /2  $\mu\text{L}$ ) and treated with a slight excess of diazomethane dissolved in diethyl ether. After 30 min, the excess diazomethane was destroyed with the addition of HCl (4M in dioxane) and the mixture was evaporated to dryness to obtain the HCl salt of compound 21c which was used in the next step without any purification.

C. To a stirred suspension of the crude dipeptide 21c (0.23 g, 0.48 mmole) in  $\text{CH}_2\text{Cl}_2$  (25 mL) was added in succession the (2S)-N-Boc-amino-hept-6-enoic acid 21d (0.151 g, 0.62 mmol), NMM (210  $\mu\text{L}$ , 1.91 mmol) and HATU (0.236 g, 0.62 mmole) and the mixture was stirred at RT for 16 h (the pH was adjusted to ~8 with NNM after 1 h if needed). The  $\text{CH}_2\text{Cl}_2$  was evaporated, the residue taken into EtOAc (50 mL) and the organic solution washed with 5%  $\text{NaHCO}_3$  (2 x 20mL), brine (2 x 20mL), dried and evaporated. The crude compound obtained was chromatographed on silica gel (50 mL, 2% EtOH/EtOAc) to give compound 21e (0.139 g, yield 46%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, rotamers in 6:1 ratio) chemical shifts of major rotamer  $\delta$

1.21-1.27 (m, 1H), 1.36 (s, 9H), 1.45-1.81 (4m, 7H), 2.20-2.22 (m, 4H), 2.28-2.37 (m, 1H), 2.90-2.99 (m, 1H), 3.66 (s, 3H), 3.94-3.98 (m, 1H), 4.29 (bd, J = 9.9 Hz, 1H), 4.46-4.50 (m, 1H), 4.81 (dd, J = 8.3 & 5.4 Hz, 1H), 4.92-5.06 (m, 4H), 5.16 (d, J = 8.3 Hz 1H), 5.37 (m, 1H), 5.70-5.84 (m, 2H), 6.82 (d, J = 5.1 Hz, 1H), 7.47-7.55 (m, 2H), 7.71 (dt, J = 7.0 & 1.3 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.78 (d, J = 5.1 Hz, 1H).

## MACROCYCLIC PEPTIDES

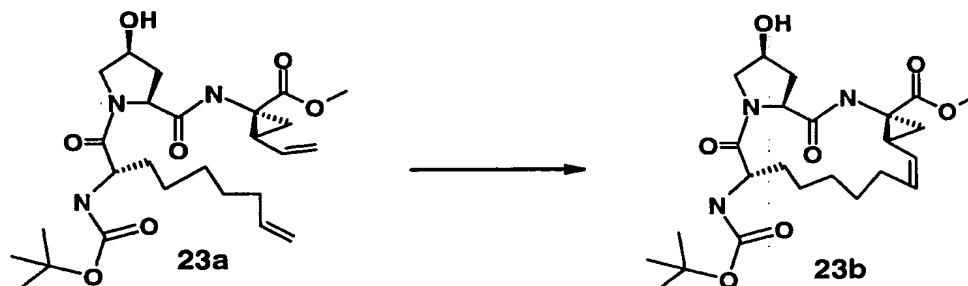
### EXAMPLE 22

#### General procedure for macrocyclization via olefin metathesis

- 10 In all cases, the tri-peptide diene was dissolved in  $\text{CH}_2\text{Cl}_2$  at a concentration of 0.01M and the solution was deoxygenated by the bubbling of argon (~1 h for a volume of 500 mL). A solution of catalyst (5-30 mol %, dissolved in a small amount of degassed  $\text{CH}_2\text{Cl}_2$ ) is added and the reaction mixture is refluxed until all starting material was converted to product(s) as indicated by TLC and HPLC. The crude
- 15 reaction mixtures were subsequently concentrated to near dryness and filtered through a short pad of silica gel, eluting first with  $\text{CH}_2\text{Cl}_2$  to remove most of the catalyst and then with EtOAc in order to elute all of the macrocyclic product(s) (most of the time as a single diastereomer). The crude product(s) from each reaction is analyzed by chiral HPLC on a CHIRALCEL OJ-R column (purchased from Chiral
- 20 Technologies Inc, 0.46  $\phi$  x 15 cm), using an isocratic solvent mixture of 70%  $\text{H}_2\text{O}$  + 0.06% TFA – 30%  $\text{CH}_3\text{CN}$  + 0.06% TFA at 205 nm. The major macrocyclic product(s) was fully characterized by:  $^1\text{H}$ , COSY, TOCSY, and ROESY NMR data in order to confirm its structure and stereochemistry.

## EXAMPLE 23

## Synthesis of macrocyclic intermediate (23b)



A solution of diene 23a (4.0 g, 7.88 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (800 mL, Aldrich-anhydrous) was deoxygenated by bubbling Ar for 2 h. Hoveyda's catalyst (262 mg, 0.434 mmol, 5.5 mol %) was then added as a solid and the reaction was refluxed under an Ar balloon. After 28 h, the red-orange solution was evaporated to an amorphous solid and then purified by flash column chromatography over silica gel.

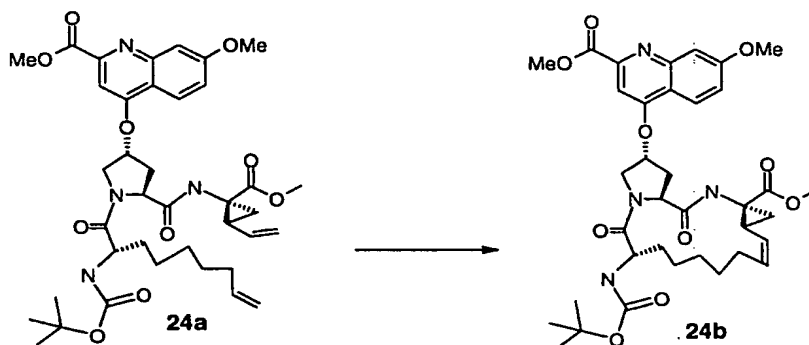
The initial solvent system was 10% EtOAc in  $\text{CH}_2\text{Cl}_2$ . Once the catalyst was eluted from the column, the solvent was changed to pure EtOAc. Elution of the catalyst from the column was evident from its color. The macrocyclic product 23b was isolated as a colorless foam which was re-dissolved in  $\text{CH}_2\text{Cl}_2$ /hexane (~1:2).

Evaporation of the solvent afforded a white powder (3.362 g, 89% yield).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.20-1.50 (m, 6H), 1.43 (s, 9H), 1.53 (dd,  $J = 9.5$  & 5.4, 1H), 1.61-1.70 (m, 1H), 1.76-1.90 (m, 2H), 2.05-2.26 (m, 4H), 2.45 (d,  $J = 14.3$ , 1H), 3.67 (s, 3H), 3.71 (d,  $J = 11.1$ , 1H), 3.90 (dd,  $J = 11.1$  & 4.3, 1H), 4.43-4.53 (m, 2H), 4.76 (d,  $J = 8.6$ , 1H), 4.86 (bd,  $J = 9.8$ , 1H), 5.20-5.23 (m, 2H), 5.57 (dt,  $J = 7.0$  & 9.8, 1H), 7.32 (bs, 1H).

## EXAMPLE 24

## Synthesis of macrocyclic intermediate (24b):

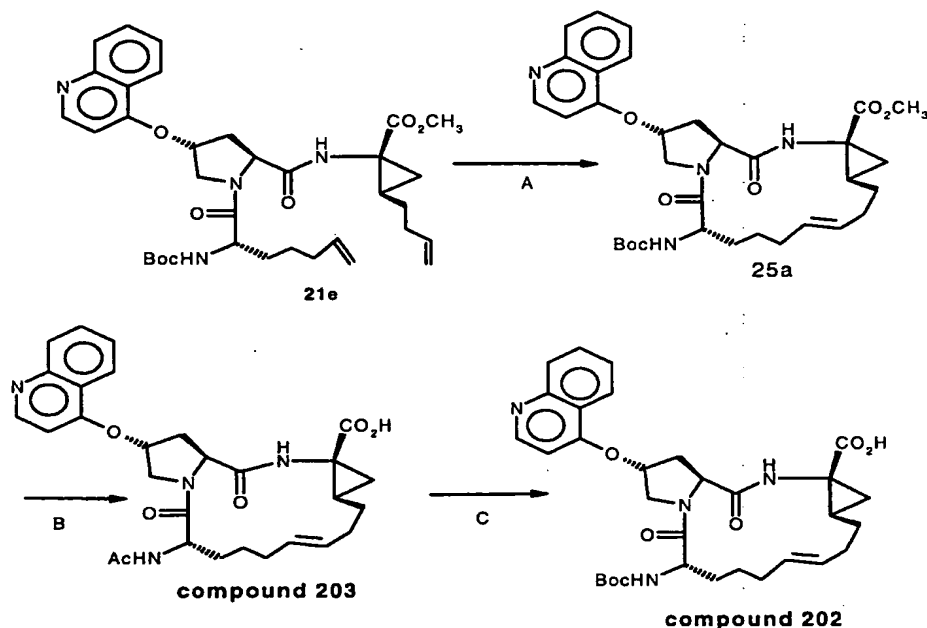


- A solution of diene **24a** (2.76 g, 3.82 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (600 mL, anhydrous) was deoxygenated by bubbling Ar for 1.5 h. A solution of Hoveyda's catalyst (117 mg, 0.19 mmol, 0.05 eq) in anhydrous and degassed  $\text{CH}_2\text{Cl}_2$  (8 mL) was added *via* cannula and the reaction was stirred at reflux under an Ar balloon. After 20 h, the reaction mixture was approximately 50% completed, at which point a second portion of catalyst was added (117 mg) and the stirring was continued for an additional 16 h. The solution was then concentrated to ~ 100 mL, applied to the top of a pad of silica gel (6 x 10 cm) and the catalyst was first recovered by eluting with  $\text{CH}_2\text{Cl}_2$ . Compound **24b** was washed off the pad of silica with 3% MeOH in EtOAc and re-purified by flash column chromatography using EtOAc/hexane (2:1) to obtain 70% yield of a slightly olive-tinted white solid (1.85 g, 94% pure by HPLC).
- $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.69 (s, 1H), 8.13 (d,  $J$  = 9.2 Hz, 1H), 7.50-7.44 (m, 2H), 7.17 (dd,  $J$  = 9.2, 2.2 Hz, 1H), 7.04 (d,  $J$  = 6.4 Hz, 1H), 5.60-5.56 (m, 1H), 5.52 (dd,  $J$  = 9.2 Hz, 1H), 5.25 (dd,  $J$  = 9.2 Hz, 1H), 4.59 (d,  $J$  = 11 Hz, 1H), 4.44 (dd,  $J$  = 9.2 Hz, 1H), 4.05-3.98 (m, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.89-3.82 (m, 1H), 3.55 (s, 3H), 2.64-2.53 (m, 1H), 2.46 (d,  $J$  = 7.3 Hz, 1H), 2.40-2.31 (m, 1H), 2.21 (dd,  $J$  = 8.9 Hz, 1H), 1.78-1.65 (m, 2H), 1.55 (dd,  $J$  = 4.8 Hz, 1H), 1.485 (dd,  $J$  = 4.8 Hz, 1H), 1.41-1.30 (m, 7H), 1.16 (s, 9H). MS;  $\text{es}^+$ : 795.4 ( $\text{M} + \text{H}$ ) $^+$ .



## EXAMPLE 25

## Synthesis of compound 202 &amp; 203 (Table 2)



- A. The diene compound 21e (0.130 g, 0.205 mmol) was cyclized using catalytic amounts of bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride (Grubb's catalyst, *supra*) (52 mg, 0.064 mmol) in  $\text{CH}_2\text{Cl}_2$  (60 mL) under reflux for 2 h to give after chromatography on silica gel (50 mL, 3% EtOH/EtOAc) compound 25a (60.1 mg, yield 48%).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.22-1.30 (m, 2H), 1.35 (s, 9H), 1.44-2.35 (m, 13H), 3.07-3.14 & 3.16-3.24 (2m, 1H, rotamers in 1:3 ratio), 3.69 (s, 3H), 3.96-4.04 (m, 1H), 4.42-4.50 (m, 1H), 4.95-5.04 (m, 1H), 5.05-5.15 (m, 1H), 5.20-5.30 (m, 1H), 5.55-5.65 (m, 1H), 6.75-6.79 (2d,  $J = 5.4$  Hz, 1H, rotamers in 1:3 ratio), 7.36 (s, 1H), 7.46-7.50 (m, 1H), 8.03 (d,  $J = 8.3$  Hz, 1H), 8.13 & 8.17 (2d,  $J = 8.0$  Hz, 1H, rotamers in 1:3 ratio), 8.77 (d,  $J = 5.1$  Hz, 1H).

- B. The ester moiety of the macrocyclic compound 25a (0.0156 g, 0.026 mmol) was hydrolyzed with  $\text{LiOH} \cdot \text{H}_2\text{O}$  (8.7 mg, 0.206 mmol) in THF/MeOH/ $\text{H}_2\text{O}$  (4 mL / 2 mL / 2 mL). The crude product was purified by C18 reversed phase HPLC on a Whatman (Partisil 10,0DS3) 50/2.4 cm column using a solvent gradient from 5%

aqueous CH<sub>3</sub>CN to 100% CH<sub>3</sub>CN to obtain pure compound 202 as an amorphous white solid (11.8 mg).

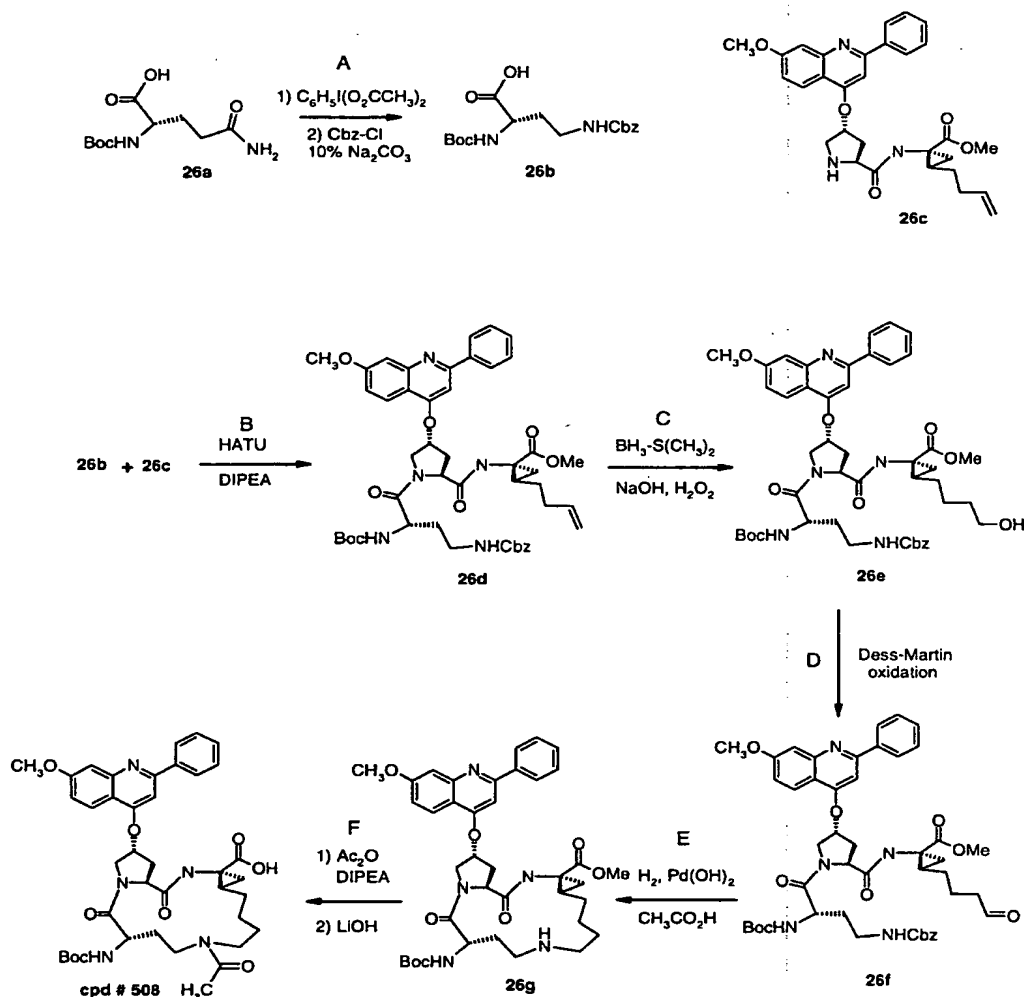
<sup>1</sup>H NMR (DMSO, 400 MHz): δ 1.12 (s, 9H), 1.20-1.24 (m, 2H), 1.32-1.40 (m, 3H), 1.58-1.62 (m, 2H), 1.68-1.78 (m, 3H), 1.95-2.02 (m, 1H), 2.08-2.18 (m, 2H), 2.42-2.59 (m, 2H), 3.97-4.00 (bd, J = 9.8 Hz, 2H), 4.47 (t, J = 8.6 Hz, 1H), 4.58 (d, J = 11.8 Hz, 1H), 5.22-5.29 (m, 1H), 5.46-5.54 (m, 1H), 5.66 (s, 1H), 7.12 (d, J = 6.0 Hz, 1H), 7.49 (d, J = 3.5 Hz, 1H), 7.68 (dd, J = 7.3 Hz, 1H), 7.98 (dd, J = 7.0 Hz, 1H), 8.08 (d, J = 8.3 Hz, 1H), 8.21 (s, 1H), 8.35 (d, J = 8.3 Hz, 1H), 9.08 (d, J = 5 Hz, 1H).

C. The macrocyclic compound 25a (20 mg, 0.033 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was stirred in presence of 4M HCl/dioxane (5 mL) for 1 h. The mixture was evaporated and dried carefully. The residue was re-dissolved in CH<sub>2</sub>Cl<sub>2</sub>/DMF (3 mL / 1 mL) and treated with NMM (14.5 μL, 0.132 mmol) and acetic anhydride (7.0 μL, 0.073 mmol) and stirred at RT for 14 h. The mixture was evaporated and dried under high vacuum. The residue was then dissolved in a mixture of THF/MeOH/H<sub>2</sub>O (4 mL / 2 mL / 2 mL) and stirred overnight with LiOH.2H<sub>2</sub>O (11 mg, 0.264 mmol). The residue isolated after acidification to pH = 3 with 1N ice-cold HCl was purified by C18 reversed phase HPLC using a solvent gradient from 0-40% aqueous CH<sub>3</sub>CN (0.06% TFA) in order to isolated pure compound 203 as an amorphous white solid (12 mg).

<sup>1</sup>H NMR (50mM Na<sub>2</sub>PO<sub>4</sub> buffer, pH=6.0, 600 MHz): δ 1.22-1.27 (m, 2H), 1.38-1.43 (m, 2H), 1.58-1.64 (m, 2H), 1.67-1.76 (m, 2H), 1.77-1.84 (m, 1H), 1.92-1.99 (m, 1H), 2.22-2.08 (m, 1H), 2.12-2.27 (m, 1H), 2.22-2.27 (m, 1H), 2.60-2.67 (m, 1H, Pro-β'), 2.83-2.89 (m, 1H, Pro-β), 4.32 (dd, J = 12.1 & 3.5 Hz, 1H, Pro-δ'), 4.41 (dd, J = 12.1 & 7.3 Hz, 1H), 4.56 (bd, J = 8.0 Hz, 1H, Pro-δ), 4.62 (dd, J = 8.9 Hz, 1H, Pro-α), 5.40-5.46 (m, 1H), 5.55-5.61 (m, 1H), 5.73 (bs, 1H, Pro-γ), 7.41 (d, J = 6.3 Hz, 1H), 7.64 (bs, 1H, AcNH), 7.80 (dd, J = 7.9 Hz, 1H), 8.03 (dd, J = 8.0 Hz, 1H), 8.07 (d, J = 9.5 Hz, 1H), 8.16 (d, J = 7 Hz, 1H, AcNH), 8.36 (d, J = 8.3 Hz, 1H), 8.90 (d, J = 6.0 Hz, 1H).

## EXAMPLE 26

## Synthesis of compound 508 (Table 5)



- A. A solution of Boc-protected L-glutamine **26a** (4.93 g, 20 mmol) and
- 5 iodobenzene diacetate (7.73 g, 24 mmol, 1.2 eq.) in EtOAc/ $\text{CH}_3\text{CN}$ / $\text{H}_2\text{O}$  (2:2:1, 60 mL), was stirred at  $16^\circ\text{C}$  for 1 h and at  $20^\circ\text{C}$  for 3 h. The reaction mixture was then diluted with  $\text{H}_2\text{O}$  (20 mL), the EtOAc and  $\text{CH}_3\text{CN}$  solvents were removed under vacuum and the remaining aqueous mixture was extracted with diethyl ether (3x50 mL) and EtOAc (50 mL) in order to remove most of the impurities. The aqueous
- 10 layer (containing the amine intermediate) was then concentrated to dryness, the

- remaining material was re-dissolved in 10% Na<sub>2</sub>CO<sub>3</sub> (30 mL), cooled to 0°C in an ice bath and a solution of benzyl chloroformate (3.3 mL, 20.4 mmol, 1.02 eq.) dioxane (40 mL) was slowly added (~10 min). The reaction mixture was stirred at 0°C for 1 h and at RT for 2 h. The mixture was then diluted with H<sub>2</sub>O (50 mL), extracted with
- 5 cold (~5°C) diethyl ether (3x50 mL), acidified with 4M HCl to pH=3-4 and extracted with EtOAc (3x50 mL). The combined organic layers were dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness under vacuum. The crude material was purified by flash column chromatography, using EtOAc/Hexane/AcOH (7:2.9:0.1) to obtain compound **26b** in 43% overall yield (3.04 g).
- 10 **B.** Dipeptide intermediate **26c** (250 mg, 0.41 mmol), compound **26b** (171 mg, 0.49 mmol, 1.2 eq.) and HATU (185 mg, 0.49 mmol, 1.2 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) and DIPEA (0.29 mL, 1.62 mmol, 4 eq.) was added. The reaction mixture was stirred at RT for 14 h, then the CH<sub>2</sub>Cl<sub>2</sub> was evaporated under vacuum and the crude material re-dissolved in EtOAc. The EtOAc solution was washed with aqueous 5%
- 15 NaHCO<sub>3</sub> and brine, dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness. Compound **26d** was obtained after purification of the crude material by flash column chromatography, using EtOAc/hexane (4:1) as the eluent, in 98% yield (338 mg).
- C.** A solution of compound **26d** (335 mg, 0.394 mmol) in THF (5 mL) was cooled
- 20 to 0°C and a solution of BH<sub>3</sub> in dimethyl sulfide (0.12 mL of 10M solution, 1.2 mmol, 3 eq.) was added. The reaction mixture was allowed to warm-up to RT and stir for 1 h. Then it was cooled again to 0°C before an aqueous solution of NaOH (0.8 mL of 2.5 M solution, 1.97 mmol, 5 eq) was added slowly over a period of 15 min, followed by the slow addition (~15 min) of an aqueous solution of H<sub>2</sub>O<sub>2</sub> (0.8 mL of an 8.8 M
- 25 solution, 6.9 mmol, 17.5 eq.). The reaction mixture was allowed to warm-up to RT and stir for 1 h. After that period, the reaction mixture was acidified to pH ~4 in order to quench the excess BH<sub>3</sub>, then aqueous NaHCO<sub>3</sub> was added to adjust the pH=~9-10, the THF was removed under vacuum and the crude material was partitioned between H<sub>2</sub>O and EtOAc. The aqueous layer was re-extracted with

## 75

EtOAc, the combined organic layers were washed with brine, dried over anhydrous  $\text{MgSO}_4$  and evaporated to dryness under vacuum. The crude material was purified by flash column chromatography, using EtOAc/hexane/ $\text{NH}_4\text{OH}$  (8:2:0.5) as the eluent, to obtain pure compound 26e in 57% yield (192 mg).

- 5 D. To a solution of compound 26e in  $\text{CH}_2\text{Cl}_2$  (8 mL), Dess-Martin periodinate (195 mg, 97%, 0.33 mmol, 1.5 eq) was added and the reaction mixture was stirred at RT for 1.5 h. The reaction was quenched with the addition of aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  (3 mL of 5% solution), then saturated aqueous  $\text{NaHCO}_3$  (5 mL) was added and the mixture was stirred at RT for 15 min. Finally, the reaction crude was extracted with
- 10 EtOAc, the organic layer was washed with aqueous 5%  $\text{NaHCO}_3$  and brine, dried over anhydrous  $\text{MgSO}_4$  and evaporated under vacuum to give 188 mg of aldehyde 28f which was used in the next step without further purification.
- E. A solution of compound 26f (188 mg, 0.22 mmol),  $\text{CH}_3\text{CO}_2\text{H}$  (38  $\mu\text{L}$ ) and  $\text{Pd}(\text{OH})_2$  (25 mg) in ethanol (5 mL) was stirred to RT under  $\text{H}_2$  at atmospheric
- 15 pressure for 16 h. After that period, more  $\text{H}_2$  gas,  $\text{Pd}(\text{OH})_2$  (180 mg) and  $\text{CH}_3\text{CO}_2\text{H}$  (154  $\mu\text{L}$ ) and were added to the flask and stirring was continued for an additional 24 h. The mixture was then filtered and the solvent evaporated to dryness, the crude macrocyclic product was purified by flash column chromatography, using  $\text{CHCl}_3/\text{MeOH}/\text{AcOH}$  (10:2:1), to obtain compound 26g in ~30% yield (48 mg).
- 20 F. A mixture of compound 26g (22 mg, 0.031 mmol), DIPEA (27  $\mu\text{L}$ , 0.155 mmol, 5 eq.) and acetic anhydride (8.7  $\mu\text{L}$ , 0.093 mmol, 3 eq.) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was stirred at RT for 16 h. The  $\text{CH}_2\text{Cl}_2$  was then removed under vacuum, a mixture of THF/MeOH/ $\text{H}_2\text{O}$  (2:2:1, 5 mL) and  $\text{LiOH}\cdot 2\text{H}_2\text{O}$  (13 mg, 0.31 mmol, 10 eq.) were added and the hydrolysis reaction was allowed to proceed for 68 h at RT and 2 h at
- 25  $50^\circ\text{C}$ . The reaction mixture was then acidified ( $\text{pH}\approx 4$ ) and purified by reversed phase HPLC to obtain the final compound 508 (~6 mg, ~26% yield for the last 2 steps).

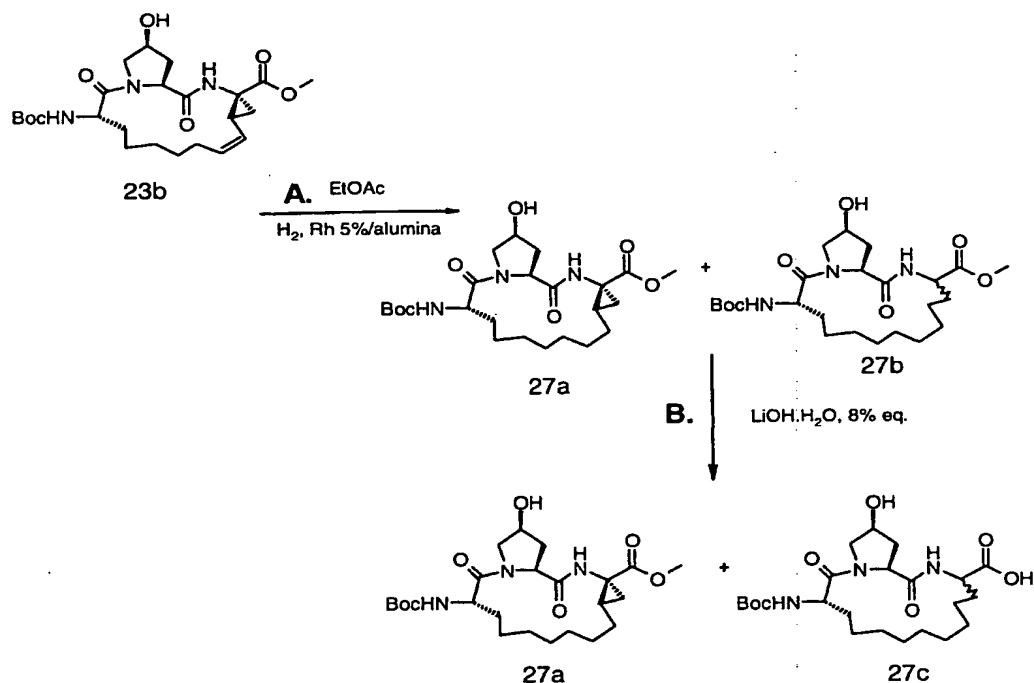
$^1\text{H}$  NMR (DMSO, 400 MHz) of 508 (mixture of rotamers confirmed by COSY,

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TOCSY and ROESY NMR data):  $\delta$  1.18 (s, 9H), 1.09-1.85 (overlapping m, 11H), 1.95 (s, 3H), 2.30 (m, 1H), 2.63 (m, 1H), 3.18-4.14 (overlapping m, 6H), 3.96 (s, 3H), 4.44 (m, 1H), 4.62 & 4.69 (2d,  $J = 11.8$  Hz, 1H, rotamers), 5.82 (bs, 1H), 7.20 (m, 2H), 7.53 (bs, 1H), 7.67 (bs, 4H), 8.19 (bs, 3H), 8.61 (s, 1H).

## 5 EXAMPLE 27

## Synthesis of the saturated macrocyclic intermediate (27a)



- A. The unsaturated macrocyclic intermediate 23b (3.50 g, 7.30 mmol) was dissolved in EtOAc (30 mL) and 700 mg (20% w/w) of 5% Rh on alumina was added. The mixture was stirred under  $H_2$  gas at atmospheric pressure and at RT for 1.5 h. After that period, HPLC analysis confirmed the complete conversion of starting material to two products, the desired product 27a and a minor product (8% of the total mass) which was later identified to be compound 27b, formed from opening of the cyclopropane ring. The reaction mixture was filtered and concentrated to give a light green color solid (3.47 g). The solid was co-evaporated twice with EtOH to remove all of the EtOAc (the presence of EtOAc interferes in the next step). Separation of compound 27a from 27b by chromatography proved to be

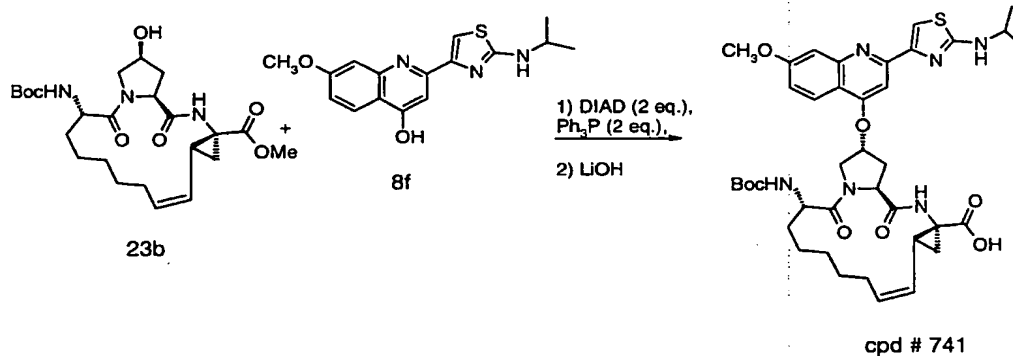
very difficult, thus an alternative method was devised based on the relative rates of hydrolysis of their respective methyl ester moieties.

- B. The crude mixture of compounds **27a** and **27b** (3.47g) was dissolved in THF:MeOH (1:1, 20 mL), an aqueous solution of LiOH•H<sub>2</sub>O (24 mg in 5 mL H<sub>2</sub>O, 8% eq) was added and the reaction mixture was stirred at RT for 16 h (complete hydrolysis of the side product **27b** to its corresponding acid **27c** was confirmed by HPLC). The reaction mixture was concentrated under vacuum in order to remove most of the THF and MeOH and partitioned between H<sub>2</sub>O (100 mL) and EtOAc (300 mL). The organic layer was washed with 0.5 N NaOH (3x100 mL), brine (100 mL), 10% aqueous citric acid (2x100 mL), brine (100 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated to dryness. The desired product **27a** was obtained in high purity (>90% by HPLC) as a light green foam and in 93% overall yield (3.28 g) for the two steps.

- <sup>1</sup>H NMR: (400 MHz, CDCl<sub>3</sub>): δ 1.1-1.38 (m, 13 H), 1.42 (s, 9 H), 1.51-1.57 (m, 1 H), 1.63-1.67 (dd, J = 8.0 & 5.1 Hz, 1 H), 1.81-1.87 (m, 1 H), 1.92-1.99 (m, 1 H), 2.02-2.08 (m, 1 H), 2.62 (d, J = 14 Hz, 1 H), 3.4 (d, J = 8.3, 1H), 3.65 (s, 3H), 4.01 (dd, J = 10.8 & 4.1 Hz, 1 H), 4.42-4.48 (m, 1 H), 4.51-4.55 (m, 1 H), 4.87 (d, J = 8.6 Hz, 1 H), 5.14 (d, J = 8.6 Hz, 1 H), 7.97 (br s, 1 H).

#### EXAMPLE 28

- 20 **Synthesis of compound #741 (Table 7)**



Quinoline derivative **8f** was attached to the pre-formed macrocyclic compound **23b** via a Mitsunobu reaction. The quinoline derivative **8f** (30 mg, 0.095 mmol) was

dissolved in THF, then macrocycle **23b** (45.6 mg, 1 eq.) and  $\text{PPh}_3$  (49.8 mg, 2 eq.) are then added. The resulting mixture is cooled to  $0^\circ\text{C}$ . DIAD (37.4  $\mu\text{L}$ , 2 eq.) is then added dropwise. The solution is stirred 1 hour at  $0^\circ\text{C}$  then stirred overnight at room temperature. The mixture was then diluted with EtOAc (15 ml), washed with a saturated solution of  $\text{NaHCO}_3$  (15 ml), followed by brine. The solution was dried with  $\text{MgSO}_4$ , filtered and concentrated in vacuo. 202 mg of a yellow oil was obtained. The product was purified by flash chromatography on silica gel (100% EtOAc). The product still contained DIAD byproducts after the purification. The resulting product obtained contained 55% w/w of the desired product, so the yield was declared to be 62%.

The ester intermediate (46 mg, 0.06 mmol) was dissolved in a mixture of THF/MeOH/ $\text{H}_2\text{O}$  (2:1:1 ratio, 2 mL),  $\text{LiOH}\cdot\text{H}_2\text{O}$  (20 mg, 0.48 mmol) was added and the solution was stirred at RT. After a period of 16 h, analysis of the reaction mixture by HPLC indicated that the hydrolysis was complete. The organic solvents were removed under vacuum and the remaining crude material dissolved in DMSO was purified by C18 reversed phase HPLC to give pure inhibitor **741**.

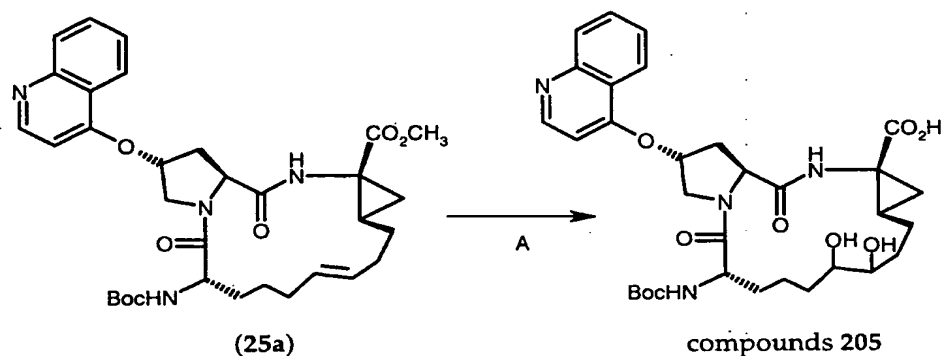
$^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  (ppm): 8.67 (s, 1H), 8.29-8.14 (m, 2H), 8.08-7.97 (m, 1H), 7.91-7.78 (m, 1H), 7.74 (s, 1H), 7.31-7.20 (m, 1H), 7.10 (d,  $J = 5.7$  Hz, 1H), 5.82-5.71 (m, 1H), 5.58-5.47 (m, 1H), 5.32-5.23 (m, 1H), 4.74-4.64 (m, 1H), 4.55-4.47 (m, 1H), 4.23-4.06 (m, 1H), 4.04-3.94 (m, 1H), 3.97 (s, 3H), 3.92-3.85 (m, 1H), 2.70-2.55 (m, 2H), 2.53-2.36 (m, 2H), 2.20-2.09 (m, 1H), 1.80-1.62 (m, 2H), 1.56-1.43 (m, 2H), 1.42-1.29 (m, 6H), 1.27 (d,  $J = 3.2$  Hz, 3H), 1.25 (d,  $J = 2.9$  Hz, 3H), 1.12 (s, 9H).

MS: 763.1 (M+1), 761.1 (M-1).



## EXAMPLE 29

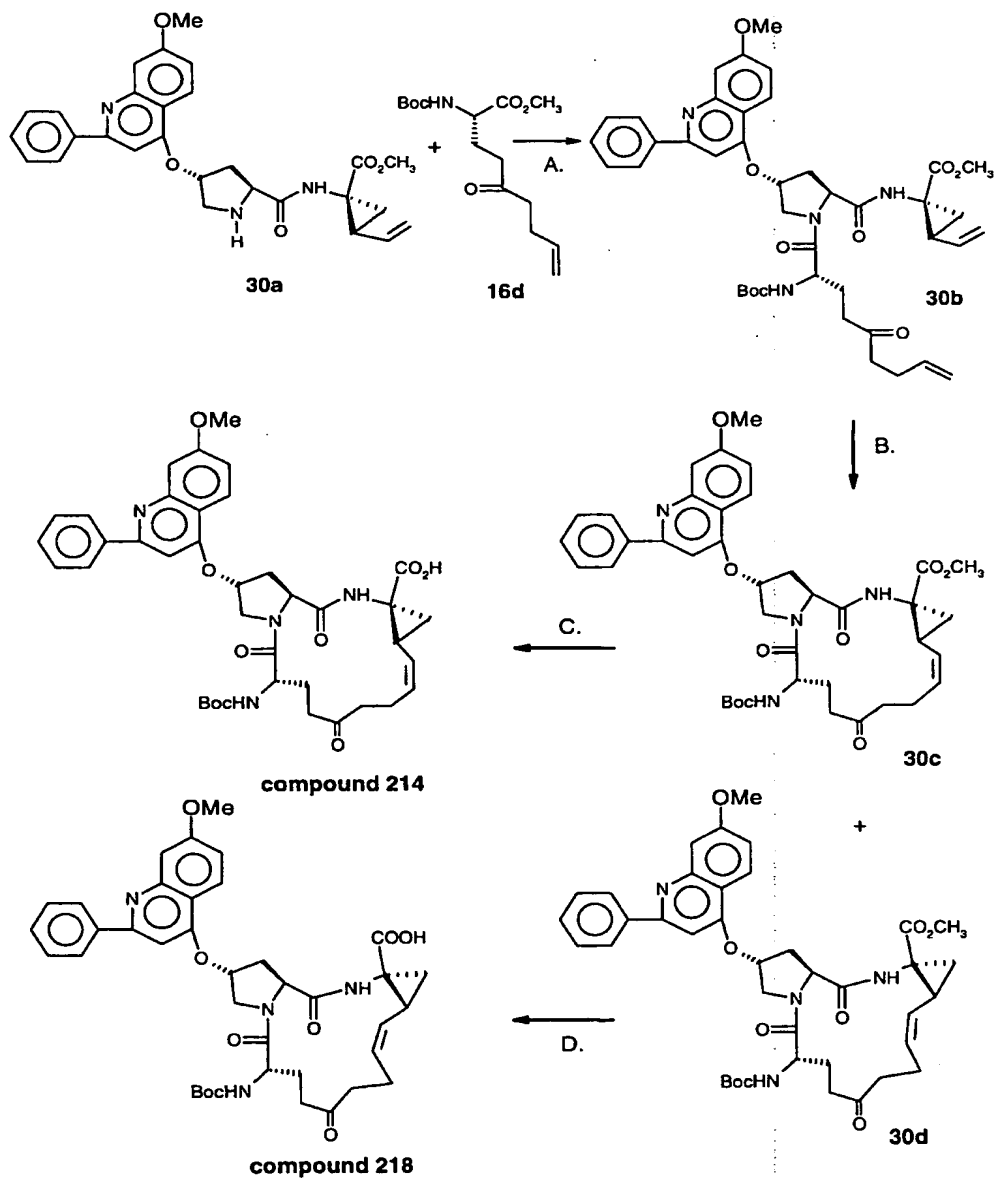
## Synthesis of compound 205 (Table 2)



- 5 To a solution of the macrocyclic compound 25a (21 mg, 0.035 mmol) in t-butanol/H<sub>2</sub>O (1.5 mL/1.5 mL) at 0°, a solution of OsO<sub>4</sub> in t-butanol (0.36 mL of a 35% w/v, 0.035 mmol) was added and the mixture was stirred at RT for 1 h. The mixture was diluted with EtOAc (20 mL) and the organic solution washed with 5% NaHCO<sub>3</sub> (2 x 10 mL), brine (2 x 10 mL), dried and evaporated to dryness. The crude
- 10 compound was taken into THF/MeOH/H<sub>2</sub>O (3 mL / 1.5 mL / 1.5 mL) and stirred in presence of LiOH•H<sub>2</sub>O (13 mg, 0.28 mmol) for 16 h. The mixture was acidified to pH 4 with 0.5 N ice-cold HCl, evaporated and purified by C18 reversed phase HPLC using a solvent gradient from H<sub>2</sub>O (0.06%TFA) to 40% aqueous CH<sub>3</sub>CN (0.06% TFA). The syn diol 205 was isolated in high purity as amorphous white solid.
- 15 compound #205: <sup>1</sup>H NMR (DMSO, 400 MHz): δ 1.01 (s, 9H), 1.06-1.30 (m, 9H), 1.48-1.68 (m, 3H), 1.78-1.88 (m, 1H), ≈2.2-2.5 (2m, 2H), 3.78-3.82 (m, 1H), 3.86-3.90 (m, 1H), 4.39 (t, J = 8.9 Hz, 1H), 4.61 (d, J = 11.4 Hz, 1H), 5.60 (bs, 1H, Pro-γ), 7.03 (d, J = 6.0 Hz, 1H), 7.40 (bs, 1H), 7.58-7.62 (m, 1H), 7.87-7.91 (m, 1H), 8.00 (d, J = 8.3 Hz, 1H), 8.24 (d, J = 8.6 Hz, 1H), 8.60 (s, 1H), 8.99 (bs, 1H).
- 20 EMS (negative ionization mode): m/z 625 (M-H)<sup>-</sup>.

## EXAMPLE 30

## Synthesis of compound 214 &amp; 218 (Table 2)



- 5 A. A solution of the keto-nonenoate ester 16d (0.180 g, 0.6 mmol) in MeOH/H<sub>2</sub>O (5 mL / 2 mL) was stirred at RT in presence of LiOH•H<sub>2</sub>O (50 mg, 1.2 mmol) for 1 h. The solution was acidified to pH 6 with 0.5 N ice-cold HCl and most of the MeOH was evaporated. The residue was then dissolved in EtOAc (30 mL)

and the solution was washed with 0.5 N ice-cold HCl (10 mL), brined (10 mL), dried and evaporated. The crude residue was then re-dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and reacted with the P1-P2 fragment 30a (0.337 g, 0.6 mmol) in the presence of HATU (233 mg, 0.612 mmol) and DIPEA (420  $\mu\text{L}$ , 2.4 mmol) over a period of 16 h at RT.

- 5 The reaction mixture was chromatographed on silica gel using EtOAc/hexane (1/1) as the eluent, to isolate the pure compound 30b (0.370 g, yield 83%, Purity >95% by HPLC).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.41 (s, 9H), 1.45-1.54 (m, 1H), 1.58-1.62 (m, 1H), 1.73-1.77 (m, 1H), 1.86-1.91 (m, 1H), 2.16 (dd,  $J = 17.8$  & 8.6 Hz, 1H), 2.26-2.43 (2m, 2H),  
10 2.46-2.58 (m, 2H), 2.64-2.81 (m, 1H), 2.85-2.92 & 2.95-3.03 (2m, 1H, rotamers in 1:3 ratio), 3.67 (s, 3H), 3.95 (s, 3H), 4.10-4.18 (m, 1H), 4.20-4.30 (m, 1H), 4.40-4.55 (m, 1H), 4.80-4.88 (m, 1H), 4.92-5.10 (m, 2H), 5.14 (dd,  $J = 10.2$  & 1.6 Hz, 1H), 5.24-5.38 (m, 4H), 5.42-5.54 (m, 1H), 5.68-5.86 (m, 2H), 7.04-7.14 (m, 2H), 7.42-7.64 (m, 5H), 7.92-8.12 (m, 3H).

- 15 B. The diene 30b (0.370 g, 0.49 mmol) was cyclized in the presence of the bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride catalyst (0.125 mg, 0.15 mmol) in  $\text{CH}_2\text{Cl}_2$  (distilled from  $\text{CaH}_2$  and degassed with argon for 30 min) over a period of 2 h at reflux. The compound was obtained as a mixture of stereoisomers (30c and 30d 1:1 ratio) after flash column chromatography on silica gel using  
20 EtOAc/Hexane (3/1) in 35% yield (0.124 g).

$^1\text{H}$  NMR of mixture 30c & 30d ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.44 (s, 4H) & 1.37 (s, 4H), 1.60 (m, 2H), 1.83 (m, 0.5H), 2.01 (m, 1H), 2.09 (m, 1H), 2.42 (m, 5H), 2.73 (m, 2H), 3.26 (m, 0.5H), 3.69 (s, 1.5H), 3.76 (s, 1.5H), 3.96 (s, 3H), 4.10 (m, 1H), 4.24 (m, 0.5H), 4.10 (m, 0.5H), 4.58 (m, 1H), 4.73 (m, 1H), 4.89 (m, 0.5H), 4.97 (m, 0.5H), 5.30 (m, 0.5H),  
25 5.44 (m, 2H), 5.64 (m, 1H), 7.1-7.0 (m, 3H), 7.47 (m, 4H), 8.08-7.98 (m, 3H).

- C,D. Hydrolysis of the methyl esters 30c and 30d (24 mg, 0.033 mmol) was carried out in THF/MeOH/ $\text{H}_2\text{O}$  (1 mL / 0.5 mL / 0.5 mL) with  $\text{LiO}\cdot\text{H}_2\text{O}$  (11 mg, 0.246 mmol) over a period of 16 h at RT. After that period the reaction mixture was acidified to pH 4-5 and chromatographed on a C18 reversed phase HPLC column  
30 using a solvent gradient from  $\text{H}_2\text{O}$  (0.06% TFA) to 50% aqueous  $\text{CH}_3\text{CN}$  (0.06% TFA). The desired compounds 214 and 218 were isolated from the mixture of the

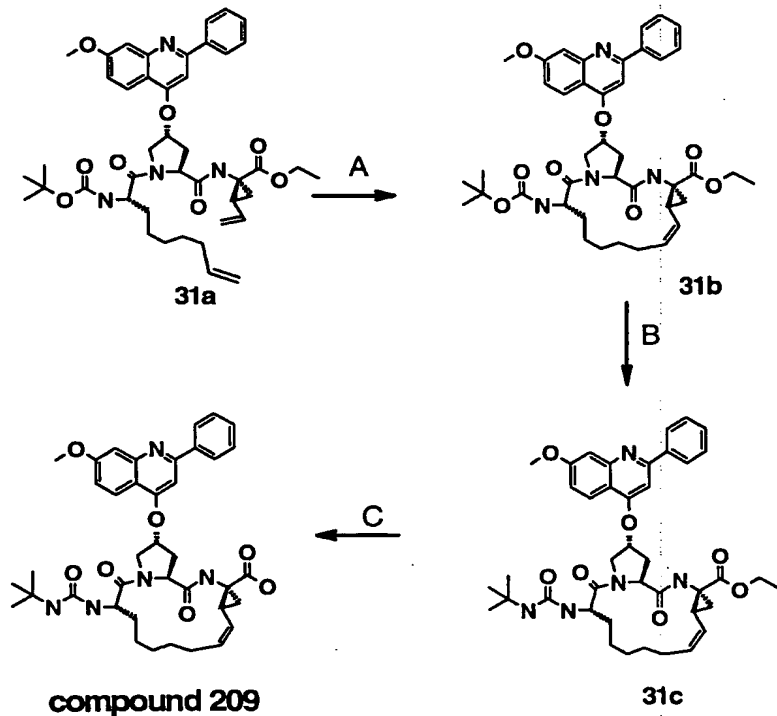
two compounds in high purity (94% pure by HPLC) in 15% yield (3 mg).

compound 214:  $^1\text{H}$  NMR (DMSO, 400 MHz)  $\delta$  1.15 (s, 9H), 1.48-1.54 (m, 2H), 1.65-1.74 (m, 1H), 1.77-1.85 (m, 1H), 2.12-2.25 (m, 4H), 2.27-2.34 (m, 1H), 2.61-2.68 (m, 1H), 2.87 (bt,  $J = 11.5$  Hz, 1H), 3.92 (dd,  $J = 9.2$  & 1.5 Hz, 1H, Pro- $\delta$ ), 3.97 (s, 3H,  $-\text{OCH}_3$ ), 4.14-4.20 (m, 1H), 4.52 (t,  $J = 7.8$  Hz, 1H, Pro- $\alpha$ ), 4.66 (d,  $J = 11.8$  Hz, 1H, Pro- $\delta$ ), 5.45 (t,  $J = 9.9$  Hz, 1H), 5.51-5.58 (m, 1H), 5.82 (bs, 1H, Pro- $\gamma$ ), 7.09 (d,  $J = 6.0$  Hz, 1H, BocNH), 7.26 (bs, 1H), 7.53 (s, 1H), 7.67 (bs, 3H), 8.16 (d,  $J = 2$  Hz, 1H), 8.18 (s, 1H), 8.83 (s, 1H, ACCA-NH).

compound 218:  $^1\text{H}$  NMR(DMSO, 400 MHz):  $\delta$  1.06-1.10 (m, 1H), 1.18 (s, 9H), 1.52-1.55 (m, 1H), 1.62-1.80 (m, 1H), 2.10-2.68 (overlapping, 9H), 3.90 (bd,  $J = 8.3$  Hz, 1H), 3.96 (s, 3H,  $\text{OCH}_3$ ), 4.20-4.27 (m, 1H), 4.58-4.63 (m, 1H, Pro- $\delta$ ), 4.66 (dd,  $J = 8.3$  Hz, 1H, Pro- $\alpha$ ) 4.88 (dd,  $J = 10.2$  Hz, 1H), 5.18-5.26 (m, 1H), 5.73-5.79 (m, 1H, Pro- $\gamma$ ), 7.01 (d,  $J = 6.4$  Hz, 1H), 7.23 (bs, 1H), 7.50 (bs, 1H), 7.66 (bs, 3H), 8.20 (bs, 2H), 8.53 (s, 1H).

#### EXAMPLE 31

##### 15 Synthesis of compound 209 (Table 2)



- A. The diene **31a** (249 mg, 0.330 mmol) was dissolved in 30 mL of anhydrous  $\text{CH}_2\text{Cl}_2$  and the solution was degassed with argon for 15 min. The catalyst bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride (82 mg, 0.100 mmol) was dissolved in 3 mL of anhydrous and degassed  $\text{CH}_2\text{Cl}_2$  and added to the diene solution. The reaction mixture was refluxed for 2 h under  $\text{N}_2$ . The solution was concentrated and purified by flash column chromatography to obtain compound **31b** as a brown solid in 71% yield (171 mg).
- $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.22-1.44 (m, 10H), 1.42 (s, 9H), 1.66-1.74 (m, 1H), 1.87-1.97 (m, 2H), 2.13-2.28 (m, 3H), 2.32-2.39 (m, 1H), 3.08-3.16 (m, 1H), 3.41 (s, 3H), 4.07-4.22 (m, 3H), 4.28-4.34 (m, 1H), 4.58-4.64 (m, 1H), 4.95-4.99 (m, 1H), 5.22-5.29 (m, 2H), 5.38-5.43 (m, 1H), 5.48-5.56 (m, 1H), 7.00-7.12 (m, 3H), 7.43-7.55 (m, 4H), 7.97-8.11 (m, 3H).
- ES(+)MS:  $m/z$  727.4 ( $\text{M}+\text{H}$ ) $^+$ .
- B. Compound **31b** (0.117 mmol) was stirred in a solution of HCl (1 mL of 4N in dioxane) for 30 min and concentrated to dryness. The solid was taken up in  $\text{CH}_2\text{Cl}_2$  (2 mL),  $\text{Et}_3\text{N}$  (82  $\mu\text{L}$ , 0.585 mmol) and *t*-butylisocyanate (35 mg, 0.351 mmol) were successively added. After stirring at RT for 20 h, the mixture was concentrated to dryness and the crude compound **31c** was used in the final hydrolysis step without further purification.
- D. Compound **31c** (85 mg, 0.117 mmol) was dissolved in THF/MeOH/ $\text{H}_2\text{O}$  (2 mL / 1 mL / 1 mL),  $\text{LiOH}\cdot\text{H}_2\text{O}$  (39 mg, 0.936 mmol) was added and the solution was stirred for 20 h at RT. After that period, acetic acid (1 mL) was added and the solution was concentrated to remove the MeOH and THF. The pure compound **209** was isolated after purification of the crude by C18 reverse phase HPLC (25 mg, ~31% yield).
- $^1\text{H}$  NMR (DMSO, 400 MHz):  $\delta$  1.04 (s, 9H), 1.15-1.24 (m, 2H), 1.30-1.40 (m, 5H), 1.44-1.51 (m, 2H), 1.54-1.68 (m, 1H), 1.75-1.88 (m, 1H), 2.18 (dd,  $J = 17.2$  & 8.5 Hz, 1H), 2.32-2.45 (m, 1H, Pro- $\beta$ ), 2.54-2.62 (m, 1H), 2.65-2.68 (m, 1H, Pro- $\beta$ ), 3.91 (dd,  $J = 11.1$  & 3.5 Hz, 1H, Pro- $\delta$ ), 3.96 (s, 3H,  $-\text{OCH}_3$ ), 4.17-4.23 (m, 1H), 4.47 (dd,  $J = 8.6$ , 1H, Pro-

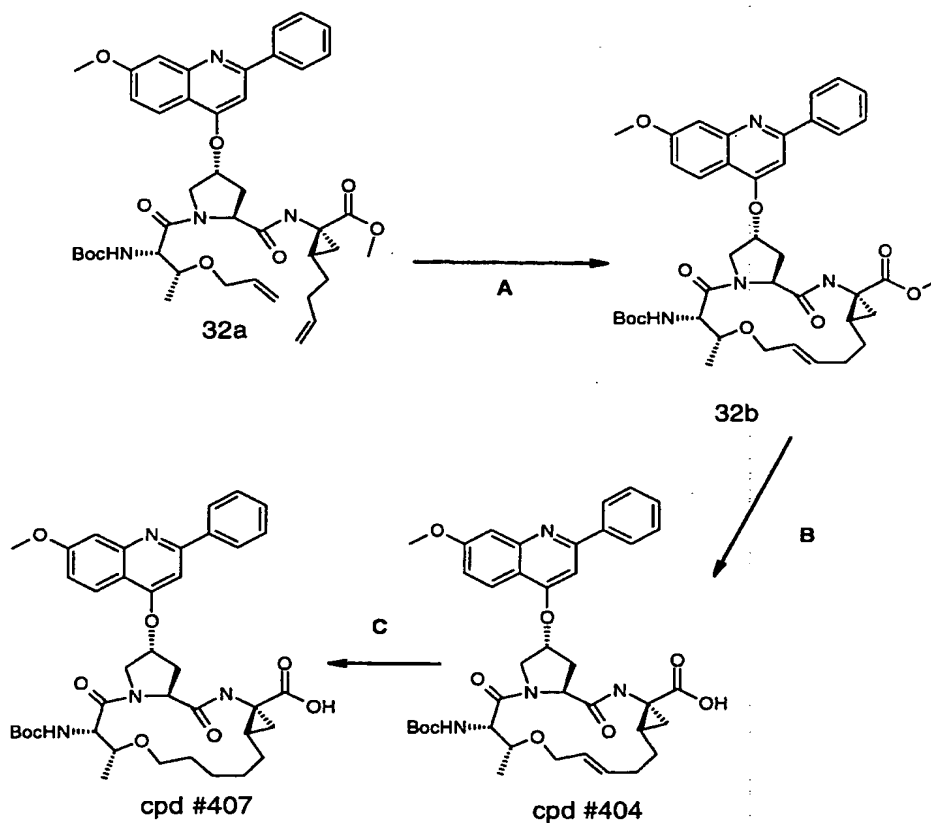
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$\alpha$ ), 4.67 (bd,  $J = 7.9$  Hz, 1H, Pro- $\delta$ ), 5.30 (dd,  $J = 9.5$  Hz, 1H), 5.52 (bdd,  $J = 19$  & 8.3, 1H), 5.68 (s, 1H), 5.78 (bs, 1H, Pro- $\gamma$ ), 5.94 (bs, 1H), 7.21 (bs, 1H), 7.51 (bs, 1H), 7.66 (bs, 4H), 8.19 (s, 2H), 8.40 (d,  $J = 7$  Hz, 1H), 8.61 (s, 1H, ACCA-NH).

ES(+)-MS:  $m/z$  698.3 ( $M+H$ )<sup>+</sup>.

## 5 EXAMPLE 32

### Synthesis of compounds #404 and #407 (Table 4)



- A. The diene 32a (84 mg, 0.11 mmol) was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (11 mL) and the solution was degassed over a period of 15 min. with a flow of argon. The bis-(tricyclohexylphosphine) benzylidene ruthenium IV dichloride catalyst (19 mg, 0.023 mmol) was first dissolved in 1 mL of degassed  $\text{CH}_2\text{Cl}_2$  and then it was transferred to the reaction flask *via* cannula. The reaction mixture was stirred for 2 h at reflux. The solvent was then removed under vacuum and the reaction mixture was purified by flash column chromatography on silica gel, using EtOAc/ hexane

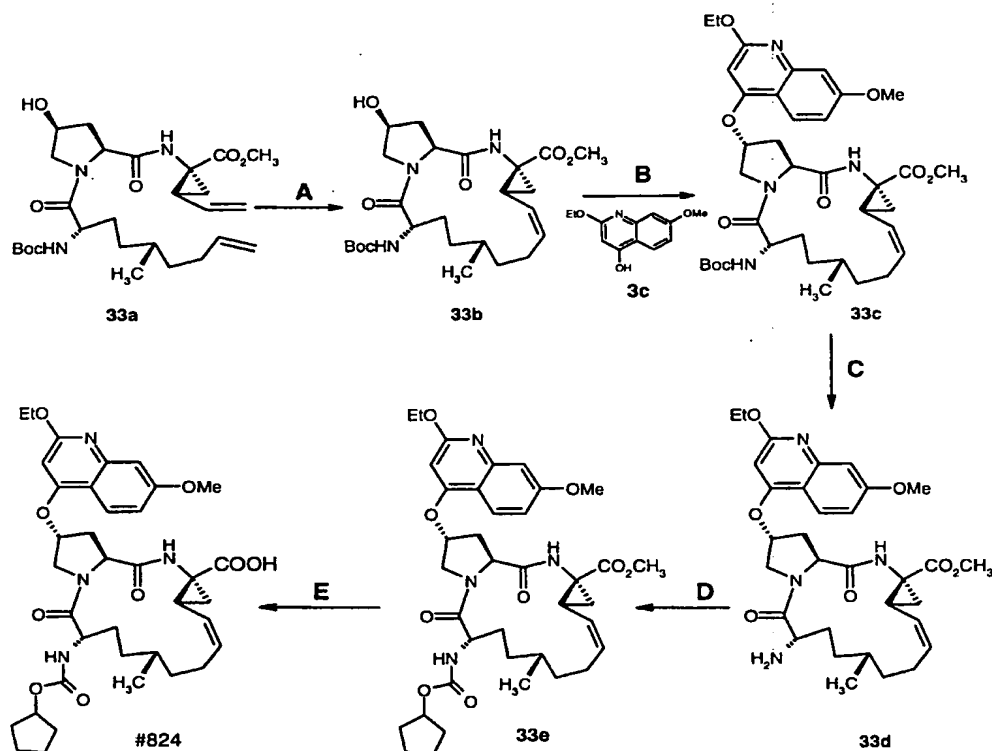
(1:1) as the eluent, to give the macrocyclic compound **32b** as a yellow oil (33 mg, 41% yield).

B. The ester intermediate **32b** (33 mg, 0.045 mmol) was dissolved in a mixture of THF/MeOH/H<sub>2</sub>O (2:1:1 ratio, 2 mL), LiOH•H<sub>2</sub>O (8 mg, 0.18 mmol) was added and the solution was stirred at RT. After a period of 16 h, analysis of the reaction mixture by HPLC indicated that the hydrolysis was incomplete. Thus an additional amount of LiOH•H<sub>2</sub>O (4 mg, 0.09 mmol) was added and the solution was stirred at RT for a total of 36 h. Finally, the solution was acidified with a small aliquot of acetic acid, the organic solvents were removed under vacuum and the remaining crude material was purified by C18 reversed phase HPLC to give pure inhibitor **404**.  
<sup>1</sup>H NMR (DMSO, 400MHz): δ 1.21 (d, J = 6.0 Hz, 3H, Me), 1.36 (s, 9H, Boc), 1.1-1.4 (3m, 3H), 1.66 (m, 1H), 1.80 (m, 1H), 2.10 (m, 2H), 2.57 (m, 2H), 3.90 (m, 4H), 4.47 (bd, J=12.7 Hz, 1H), 4.58 (bd, J=7.3, 1H), 4.66 (dd, J=8.0 Hz, 1H), 5.57 (m, 1H), 5.66 (m, 1H), 5.83 (bs, 1H), 6.18 (bd, J=6.9 Hz, 1H), 7.25 (bd, J=7.3 Hz, 1H), 7.56 (bs, 1H), 7.70 (m, 4H), 8.22 (bd, J=2.9 Hz, 2H), 8.29 (bs, J=9.2 Hz, 1H).

C. Inhibitor **404** (15 mg, 0.021 mmol) was dissolved in ethanol (2 mL) and Pd 10%/C (2 mg) was added. The mixture was stirred under hydrogen at RT for 16 h. After filtration, the mixture was purified by C18 reversed phase HPLC to give inhibitor **407** as a white solid (10 mg, 66% yield)  
<sup>1</sup>H NMR (DMSO, 400MHz): δ 1.04 (m, 1H), 1.17 (d, J = 6.0 Hz, 3 H), 1.35 (s, 9H), 1.25-1.75 (m, 12 H), 2.32-2.45 (m, 1 H), 3.40-3.50 (m, 2 H), 3.74-3.83 (m, 1H), 3.85-3.93 (m, 1H), 3.97 (s, 3H), 4.27-4.36 (dd, J = 21.1 & 8.6 Hz, 1H), 4.54 (dd, J = 7.95 & 7.95 Hz, 1H), 5.64 (d, J = 8.3 Hz, 1H), 5.82 (br s, 1H), 7.27-7.33 (m, 1H), 7.53-7.57 (bs, 1 H), 7.60-7.74 (m, 4 H), 8.13-8.27 (m, 3 H), 8.30-8.35 (br s, 1H).

## EXAMPLE 33

## Synthesis of compound #824 (Table 8)



- A. Compound 33a (~0.55 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (100 mL) and the solution was degassed carefully before a sample of Hoveyda's catalyst (17 mg, 0.028 mmol, 0.05 eq.) was added. The solution was then stirred under reflux for 5 h. The reaction mixture was concentrated and purified by flash column chromatography, using a solvent gradient of  $\text{CH}_2\text{Cl}_2$ /EtOAc (from 3:2 to 2:3 ratio), to give compound 33b in 72% yield (194 mg).
- 10 B. To a solution of compound 33b (70 mg, 0.142 mmol), 2-ethoxy-4-hydroxy-7-methoxyquinoline 3c (63 mg, 0.284 mmol, 2 eq.) and  $\text{Ph}_3\text{P}$  (186 mg, 0.71 mmol, 5 eq.) in anhydrous THF (15 mL) at 0 °C, DIAD (140  $\mu\text{L}$ , 0.71 mmol, 5 eq.) was added slowly over a period of 20 min. The reaction mixture was allowed to warm-up to RT and to stir at RT for 2.5 h. Subsequently, the THF was evaporated under vacuum
- 15 and the crude product was purified by flash column chromatography, using a



solvent gradient of hexane/EtOAc (from 7:3 to 1:1 ratio). Pure compound 33c was isolated in 73% yield (72 mg).

C. Compound 33c (72 mg, 0.104 mmol) was mixed with  $\text{CH}_2\text{Cl}_2$  (5 mL) and 4M HCl in dioxane (5 mL) and the mixture was allowed to stir at RT for 1.5 h in order to  
5 cleave the Boc protecting group and obtain the HCl salt of intermediate 33d. The reaction crude reaction mixture was evaporated to dryness under vacuum, dried under vacuum to assure the removal of all HCl and used in the next step without purification.

D. To a solution of cyclopentanol (29  $\mu\text{L}$ , 0.32 mmol) in THF (10 mL), a solution  
10 of phosgene in toluene (1.93 M, 274  $\mu\text{L}$ , 0.528 mmol) was added dropwise and the mixture was stirred at R.T. for 2 h to form the cyclopentyl chloroformate reagent. After that period, approximately half of the solvent was removed by evaporation under vacuum, the remaining light yellow solution was diluted by the addition of  $\text{CH}_2\text{Cl}_2$  (5 mL) and reconcentrated to half of its original volume, in order to assure  
15 the removal of all excess phosgene. The above solution of the cyclopentyl chloroformate reagent was further diluted with THF (10 mL), cooled to 0 °C and added to the solid compound 33d (0.104 mmol) at 0 °C.  $\text{Et}_3\text{N}$  (75  $\mu\text{L}$ , 0.534 mmol, 5.2 eq.) was added to the reaction mixture and stirring was continued at 0 °C for 1.5 h. The solvents were removed under vacuum and the crude material purified by flash  
20 column chromatography, using EtOAc/hexane (1:1) as the eluent, to obtain compound 33e in almost quantitative yield (75 mg).

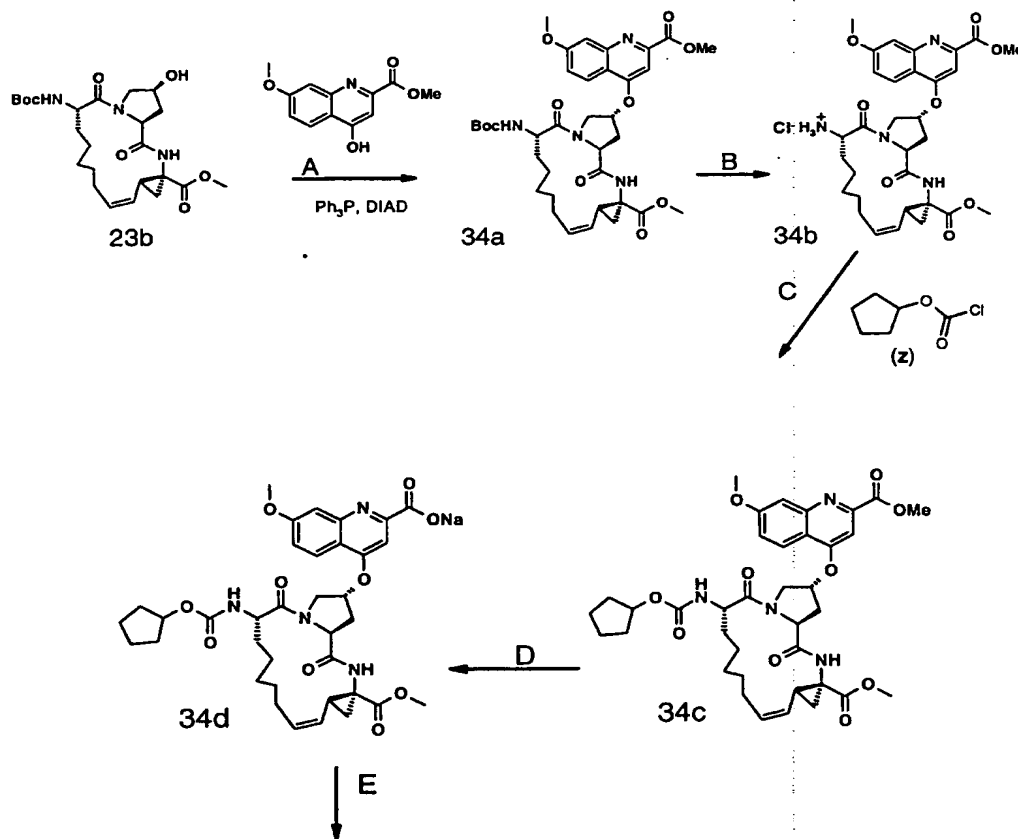
E. Hydrolysis of the methyl ester was achieved by reacting compound 33e (75 mg, 0.11 mmol) with  $\text{LiOH}\cdot\text{H}_2\text{O}$  (35 mg, 0.84 mmol, 8 eq.) in a solvent mixture of THF/MeOH/ $\text{H}_2\text{O}$  (2:2:1 ratio, 7.5 mL) at 50 °C for 2.5 h. Upon completion of the  
25 hydrolysis, the mixture was acidified to pH=4.5 and the solvents were evaporated to dryness under vacuum. The crude product was purified by C18 reversed phase preparative HPLC, using a solvent gradient of  $\text{H}_2\text{O}$  to 58% aqueous  $\text{CH}_3\text{CN}$  (with 0.06%TFA), to obtain inhibitor #824 as a white amorphous solid (45 mg, 65% yield).  $^1\text{H}$  NMR of the  $\text{Na}^+$  salt of #824 (DMSO, 400 MHz):  $\delta$  0.88 (d,  $J=6.7$  Hz, 3H), 0.95-1.70  
30 (overlapping resonances, 17H), 1.37 (t,  $J=7$  Hz, 3H), 2.00-2.10 (m, 1H), 2.10-2.33 (m,

88

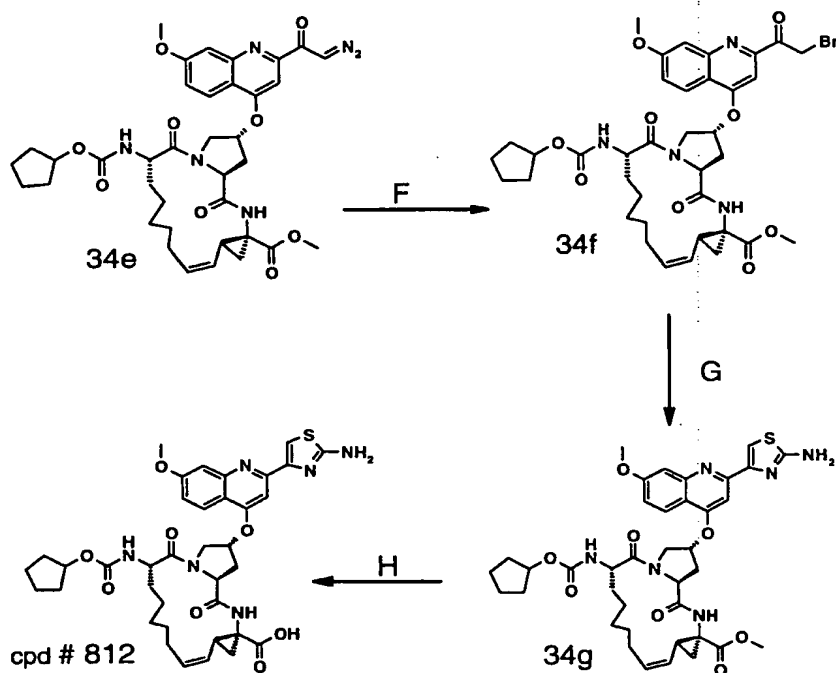
3H), 2.38-2.44 (m, 1H), 3.80-3.85 (m, 1H), 3.85 (s, 3H), 4.02-4.08 (m, 1H), 4.42 (q, J=7 Hz, 2H), 4.35-4.44 (m, 1H), 4.50 (d, J=10.8 Hz, 1H), 4.63 (bs, 1H), 5.28 (dd, J=9.5 Hz, 1H), 5.38 (bs, 1H), 5.42-5.49 (m, 1H), 6.37 (s, 1H), 6.87 (dd, J=8.9 & 2.2 Hz, 1H), 7.07 (d, J=2.2 Hz, 1H), 7.28 (d, J=7.0 Hz, 1H), 7.90 (d, J=8.9 Hz, 1H), 8.57 (s, 1H).

## 5 EXAMPLE 34

## Synthesis of compound #812 (Table 8)



89



- A. To a solution of the macrocyclic intermediate 23b (13.05 g, 27.2 mmol, 1.0 eq.), Ph<sub>3</sub>P (14.28 g, 54.4 mmol, 2.0 eq) and 2-carboxymethoxy-4-hydroxy-7-methoxyquinoline (WO 00/09543 & WO 00/09558) (6.67 g, 28.6 mmol, 1.05 eq) in
- 5 THF (450 mL) at 0°C, DIAD (10.75 mL, 54.6 mmol, 2.0 eq) was added dropwise over a period of 15 min. The ice bath was then removed and the reaction mixture was stirred at RT for 3 h. After the complete conversion of starting material to products, the solvent was evaporated under vacuum, the remaining mixture diluted with EtOAc, washed with saturated NaHCO<sub>3</sub> (2x) and brine (1x), the organic layer was
- 10 dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated to dryness. Pure compound 34a was obtained after flash column chromatography; the column was eluted first with hexane/EtOAc (50:50), followed by CHCl<sub>3</sub>/EtOAc (95:5) to remove Ph<sub>3</sub>PO and DIAD byproducts and elution of the impurities was monitored by TLC. Finally, the desired product 34a was eluted from the column with CHCl<sub>3</sub>/EtOAc (70:30).
- 15 Usually, the chromatography step had to be repeated 2-3 times before compound 34a could be isolated in high purity as a white solid with an overall yield of 68%

(12.8 g, 99.5% pure by HPLC).

- B. To a solution of the Boc-protected intermediate **34a** (1.567g) in  $\text{CH}_2\text{Cl}_2$  (15 mL), 4N HCl in dioxane (12 mL) was added and the reaction mixture was stirred at RT for 1 h. [In the event that a thick gel would form half way through the reaction  
5 period, an additional 10 mL  $\text{CH}_2\text{Cl}_2$  was added.] Upon completion of the deprotection the solvents were evaporate to dryness to obtain a yellow solid and a paste like material. The mixture was redissolved in approximately 5% MeOH in  $\text{CH}_2\text{Cl}_2$  and re-evaporated to dryness under vacuum to obtain compound **34b** as a yellow solid, which was used in the next step without any purification.
- 10 C. To a solution of cyclopentanol (614  $\mu\text{L}$ , 6.76 mmol) in THF (15 mL), a solution of phosgene in toluene (1.93 M, 5.96 mL, 11.502 mmol) was added dropwise and the mixture was stirred at R.T. for 2 h to form the cyclopentyl chloroformate reagent (**z**). After that period, approximately half of the solvent was removed by evaporation under vacuum, the remaining light yellow solution was diluted by the  
15 addition of  $\text{CH}_2\text{Cl}_2$  (5 mL) and concentrated to half of its original volume, in order to assure the removal of all excess phosgene. The above solution of the cyclopentyl chloroformate reagent was further diluted with THF (15 mL) and added to the amine-2HCl salt **34b**. The mixture was cooled to  $0^\circ\text{C}$  in an ice bath, the pH was adjusted to ~8.5-9 with the addition of  $\text{Et}_3\text{N}$  (added dropwise) and the reaction  
20 mixture was stirred at  $0^\circ\text{C}$  for 1 h. After that period, the mixture was diluted with EtOAc, washed with water (1x), saturated  $\text{NaHCO}_3$  (2x),  $\text{H}_2\text{O}$  (2x) and brine (1x). The organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated under vacuum to obtain a yellow-amber foam. Compound **34c** was obtained as a white foam after purification by flash column chromatography (using a solvent gradient  
25 from 30% hexane to 20% hexane in EtOAc as the eluent) in 80% yield (1.27 g) and >93% purity.
- D. The dimethyl ester **34c** (1.17g) was dissolved in a mixture of THF/MeOH/ $\text{H}_2\text{O}$  (20 mL, 2:1:1 ratio), and an aqueous solution of NaOH (1.8 mL, 1N, 1 eq.) was added. The reaction mixture was stirred at RT for 1 h before it was  
30 evaporated to dryness to obtain the sodium salt **34d** as a white solid (~1.66 mmol).

Compound **34d** was used in the next step without purification.

E. The crude sodium salt **34d** (1.66 mmol) was dissolved in THF (17 mL),  $\text{Et}_3\text{N}$  was added and the mixture was cooled to  $0^\circ\text{C}$  in an ice bath. Isobutylchloroformate (322  $\mu\text{L}$ , 2.5 mmol) was added dropwise and the mixture was stirred at  $0^\circ\text{C}$  for 75  
5 min. After that period, diazomethane (15 mL) was added and stirring was continued at  $0^\circ\text{C}$  for 30 min and then at RT for an additional 1 h. Most of the solvent was evaporated to dryness under vacuum, the remaining mixture was diluted with EtOAc, washed with saturated  $\text{NaHCO}_3$  (2x),  $\text{H}_2\text{O}$  (2x) and brine (1x), dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated to dryness to obtain compound **34e** as a  
10 light yellow foam (1.2g, ~1.66 mmol). The diazoketone intermediate **34e** was used in the next step without purification.

F. The diazoketone **34e** (1.2g, 1.66 mmol) dissolved in THF (17 mL) was cooled to  $0^\circ\text{C}$  in an ice bath. A solution of aqueous HBr (48%, 1.24 mL) was added dropwise and the reaction mixture was stirred at  $0^\circ\text{C}$  for 1 h. The mixture was then  
15 diluted with EtOAc, wash with saturated  $\text{NaHCO}_3$  (2x),  $\text{H}_2\text{O}$  (2x) and brine (1x), the organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated to dryness to obtain the  $\beta$ -bromoketone intermediate **34f** as a light yellow foam (~1.657 mmol).

G. To a solution of the bromoketone **34f** (600 mg, 0.779 mmol) in isopropanol (5 mL), thiourea (118 mg, 1.55 mmol) was added and the reaction mixture was placed  
20 in a pre-heated oil bath at  $75^\circ\text{C}$  where it was allowed to stir for 1 hr. The isopropanol was then removed under vacuum and the product dissolved in EtOAc (100 mL). The solution was washed with saturated  $\text{NaHCO}_3$  and brine, the organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated to afford the crude product **34g** (522 mg) as a red-brown solid. This material was used in the final step  
25 without any further purification.

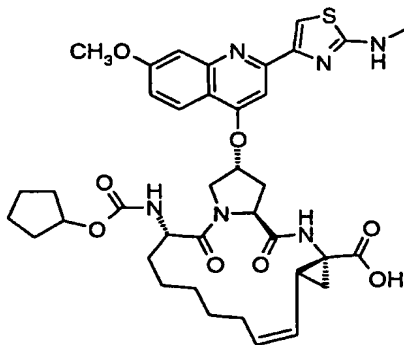
H. The crude methyl ester **34g** (122 mg, 0.163 mmol) was dissolved in a solution of THF/MeOH/ $\text{H}_2\text{O}$  (2:1:1 ratio, 4 mL) and saponified using  $\text{LiOH}\cdot\text{H}_2\text{O}$  (89 mg, 2.14 mmol). The hydrolysis reaction was carried out over a 12-15 h period at RT. The

solvents were then removed under vacuum and the crude product purified by C18 reversed phase HPLC, using a solvent gradient from 10% CH<sub>3</sub>CN in H<sub>2</sub>O to 100% CH<sub>3</sub>CN, to afford the HCV protease inhibitor #812 as a yellow solid (24 mg, 20% overall yield for the conversion of intermediate 34f to inhibitor #812).

- 5 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.63 (s, 1H), 8.26-8.15 (m, 2H), 7.79 (bs, 1H), 7.72 (bs, 1H), 7.50 (bs, 2H), 7.33-7.25 (m, 2H), 5.77 (bs, 1H), 5.52 (dd, J = 8.3 Hz, 1H), 5.27 (dd, J = 9.2 Hz, 1H), 4.64 (d, J = 10.8 Hz, 1H), 4.50 (dd, J = 8.3 Hz, 1H), 4.39-4.31 (m, 1H), 4.08-3.99 (m, 2H), 3.94 (s, 3H), 3.87 (d, J = 9.5 Hz, 2H), 2.65-2.53 (m, 2H), 2.46-2.36 (m, 2H), 2.20-2.12 (dd, J = 8.6 Hz, 1H), 1.80-1.64 (m, 2H), 1.63-1.06 (m, 14H). MS; es<sup>+</sup>: 733.2 (M + H)<sup>+</sup>, es<sup>-</sup>: 731.2 (M - H)<sup>-</sup>.
- 10

#### EXAMPLE 34A

Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-methylthiourea gave # 811 (Table 8)

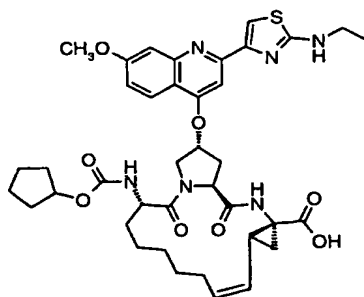


- 15 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.63 (s, 1H), 8.20 (s, 1H), 8.18 (s, 1H), 8.12-7.93 (m, 1H), 7.88-7.69 (m, 2H), 7.32-7.24 (m, 2H), 5.82-5.75 (m, 1H), 5.52 (ddd, J = 8.1 Hz, 1H), 5.28 (dd, J = 9.9 Hz, 1H), 4.67-4.61 (m, 1H), 4.51 (dd, J = 8.8 Hz, 1H), 4.44-4.37 (m, 1H), 4.08-4.00 (m, 1H), 3.96 (s, 3H), 3.89 (m, 1H), 3.04 (d, J = 4.1 Hz, 3H), 2.65-2.37 (m, 3H), 2.16 (m, 1H), 1.77-1.65 (m, 2H), 1.63-1.11 (m, 17H). MS; es<sup>+</sup>: 747.2 (M + H)<sup>+</sup>, es<sup>-</sup>: 745.3 (M - H)<sup>-</sup>.
- 20

#### EXAMPLE 34B

Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-ethylthiourea gave # 810 (Table 8)

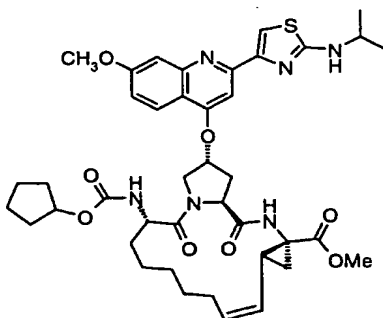
93



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.63 (s, 1H), 8.27 (bs, 1H), 8.20 (d, J = 9.0 Hz, 1H), 8.13-8.07 (m, 1H), 7.86 (bs, 1H), 7.78 (s, 1H), 7.33-7.25 (m, 2H), 5.81 (bs, 1H), 5.54 (dd, J = 8.8 Hz, 1H), 5.28 (dd, J = 9.7 Hz, 1H), 4.65 (d, J = 12.4 Hz, 1H), 4.51 (dd, J = 8.8 Hz, 1H), 4.38 (bs, 1H), 4.03 (m, 1H), 3.97 (s, 3H), 3.92-3.87 (m, 1H), 3.54-3.46 (m, 2H), 2.68-2.65 (m, 2H), 2.47-2.38 (m, 1H), 2.15 (dd, J = 8.6 Hz, 1H), 1.78-1.65 (m, 2H), 1.60-1.12 (m, 17H), 1.25 (t, J = 7.3 Hz, 3H). MS; es<sup>+</sup>: 783.2 (M + Na)<sup>+</sup>, es<sup>-</sup>: 761.2 (M + H)<sup>-</sup>.

**EXAMPLE 34C**

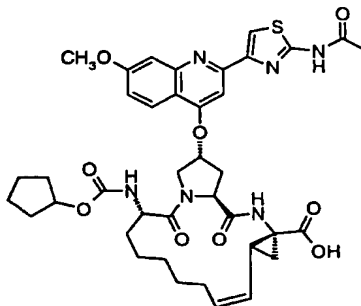
- 10 Using the same procedure as described in example 34 but reacting bromoketone 34f with commercially available *N*-iso-propylthiourea gave # 822



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.63 (s, 1H), 8.33-8.23 (bs, 1H), 8.21 (d, J = 9.2 Hz, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.86 (bs, 1H), 7.77 (s, 1H), 7.35-7.23 (m, 2H), 5.81 (bs, 1H), 5.52 (dd, J = 8.5 Hz, 1H), 5.27 (dd, J = 9.2 Hz, 1H), 4.65 (d, J = 11.8 Hz, 1H), 4.51 (dd, J = 7.6 Hz, 1H), 4.37 (bs, 1H), 4.15 (bs, 1H), 4.07-3.98 (m, 2H), 3.97 (s, 3H), 3.88 (d, J = 8.9 Hz, 1H), 2.60-2.53 (m, 2H), 2.47-2.37 (m, 2H), 2.19-2.10 (dd, J = 9.2 Hz, 1H), 1.80-1.64 (m, 2H), 1.63-1.29 (m, 13H), 1.27 and 1.25 (2 x d, J = 6.5 Hz, 6H), 1.23-1.09 (m, 2H). MS; es<sup>+</sup>: 775.0 (M + H)<sup>+</sup>, es<sup>-</sup>: 772.9 (M - H)<sup>-</sup>.

**EXAMPLE 34D**

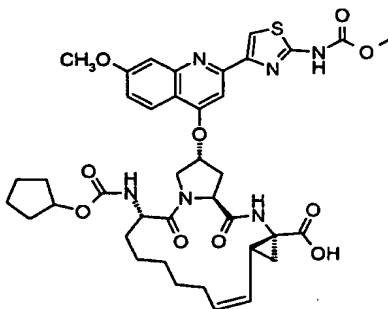
Using the same procedure as described in example 34 but reacting bromoketone **34f** with commercially available *N*-acetylthiourea gave # **809**



- 5 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.62 (s, 1H), 8.30 (bs, 1H), 8.17 (d, J= 8.9 Hz, 1H), 7.62 (bs, 1H), 7.52 (bs, 1H), 7.28 (d, J= 6.4Hz, 1H), 7.21 (bs, 1H), 5.63 (bs, 1H), 5.54 (dd, J= 8.1 Hz, 1H), 5.28 (dd, J= 9.5Hz, 1H), 4.62 (d, J= 12.1 Hz, 1H), 4.56-4.46 (m, 2H), 4.11-4.04 (m, 1H), 3.95 (s, 3H), 3.93-3.88 (m, 1H), 2.62-2.54 (m, 1H), 2.45-2.36 (m, 1H), 2.22 (s, 3H), 2.21-2.13 (m, 1H), 1.79-1.69 (m, 2H), 1.65-1.30 (m, 16H), 1.26-1.12 (m, 2H). MS; es<sup>+</sup>: 775.3 (M + H)<sup>+</sup>, es<sup>-</sup>: 773.3 (M - H)<sup>-</sup>.
- 10

**EXAMPLE 34E**

- To a stirred solution of the 2-amino-4-thiazolyl intermediate **34g** (0.24 g, 0.32 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at RT was added DIPEA (0.55 mL, 3.18 mmol, 10 eq) and methyl chloroformate (0.13 mL, 1.6 mmol, 5 eq). The reaction mixture was stirred for 6.5 h
- 15 before being concentrated under vacuum. The crude isolated material was then hydrolyzed to the desired carboxylic acid as described in Example 34 to yield compound # **818**



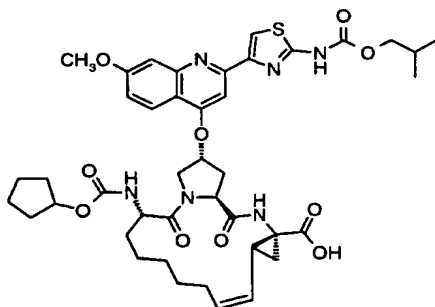
- <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.61 (s, 1H), 8.21-8.07 (m, 2H), 7.61-7.38 (m, 2H), 7.26 (d, J = 6.4 Hz, 1H), 7.19-7.10 (m, 1H), 5.60-5.47 (m, 2H), 5.27 (dd, J = 9.2 Hz,
- 20



1H), 4.63-4.53 (m, 1H), 4.47 (d,  $J = 7.9$  Hz, 1H), 4.13-4.04 (m, 1H), 3.93 (s, 3H), 3.92-3.87 (m, 2H), 3.79 (s, 3H), 2.42-2.30 (m, 2H), 2.17 (dd,  $J = 9.2$  Hz, 1H), 1.81-1.68 (m, 2H), 1.63-1.29 (m, 16H), 1.23-1.10 (m, 2H). MS;  $es^+$ : 791.1 ( $M + H$ ) $^+$ ,  $es^-$ : 789.1 ( $M - H$ ) $^-$ .

**EXAMPLE 34F**

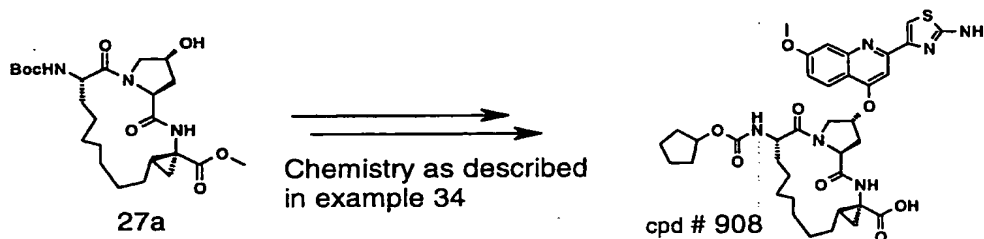
- 5 Following the conditions described above in example 34E, but using isobutyl chloroformate, gave the analogous substituted carbamate intermediate. The crude isolated material was then hydrolyzed to the desired compound # 819



- $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.62 (s, 1H), 8.47-8.27 (bs, 1H), 8.18 (d,  $J = 8.6$  Hz, 1H), 7.69-7.60 (m, 1H), 7.60-7.51 (m, 1H), 7.28 (d,  $J = 6.7$  Hz, 1H), 7.28-7.19 (m, 1H), 5.70-5.60 (m, 1H), 5.52 (dd,  $J = 8.3$  Hz, 1H), 5.27 (dd,  $J = 9.8$  Hz, 1H), 4.63 (d,  $J = 11.8$  Hz, 1H), 4.53-4.44 (m, 2H), 4.10-3.99 (m, 1H), 4.04 (d,  $J = 6.7$  Hz, 2H), 3.95 (s, 3H), 3.94-3.87 (m, 1H), 2.65-2.53 (m, 1H), 2.46-2.34 (m, 1H), 2.16 (dd,  $J = 8.1$  Hz, 1H), 2.03-1.91 (m, 1H), 1.79-1.09 (m, 20H), 0.95 (d,  $J = 6.7$  Hz, 6H). MS;  $es^+$ : 833.2 ( $M + H$ ) $^+$ ,  $es^-$ : 831.2 ( $M - H$ ) $^-$ .

**EXAMPLE 35****Synthesis of compound #908**

Starting with derivative 27a and using the same chemistry as described in example 34, the following saturated macrocycle, compound # 908 (Table 9) was obtained.



20

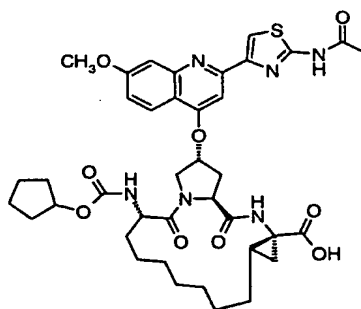
$^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.47 (s, 1H), 8.16 (d,  $J = 10$  Hz, 1H), 8.15-8.07 (m,

1H), 7.82-7.63 (m, 2H), 7.53-7.43 (m, 2H), 7.33-7.22 (m, 1H), 7.13 (d,  $J = 7$  Hz, 1H), 5.77-5.65 (m, 1H), 4.62-4.52 (m, 2H), 4.50-4.4 (m, 1H), 4.20-4.10 (m, 1H), 3.94 (s, 3H), 3.89-3.83 (m, 1H), 2.59-2.53 (m, 1H), 2.48-2.40 (m, 1H), 1.79-1.0 (m, 25H);). MS;  $es^+$ : 735.2 ( $M + H$ ) $^+$ ,  $es^-$ : 733.2 ( $M - H$ ) $^-$ .

## 5 EXAMPLE 35A

### Synthesis of compound #909

Using the same procedure as described in example 35 but using available *N*-acetylthiourea gave compound # 909 (Table 9).

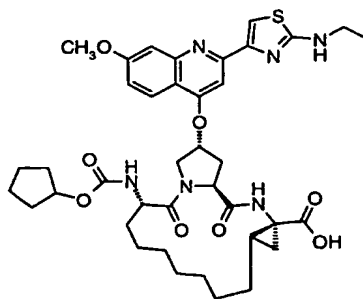


<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.53-8.41 (m, 2H), 8.20 (d, J = 9.2 Hz, 1H), 7.68 (bs, 1H), 7.68 (bs, 1H), 7.27 (dd, J = 9.2 Hz, 1H), 7.15 (d, J = 6.4 Hz, 1H), 5.67 (bs, 1H), 4.65-4.50 (m, 3H), 4.44-4.37 (m, 1H), 4.21-4.13 (m, 1H), 3.96 (s, 3H), 3.99-3.86 (m, 1H), 2.62-2.39 (m, 2H), 2.24 (s, 3H), 1.78-1.67 (m, 3H), 1.67-1.01 (m, 22H).  
MS; es<sup>+</sup>: 798.0 (M + Na)<sup>+</sup>, es<sup>+</sup>: 777.0 (M + H)<sup>+</sup>.

**15      EXAMPLE 35B**

### Synthesis of compound #910

Using the same procedure as described in example 35 but using available *N*-ethylthiourea gave compound # 910 (Table 9).



20 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.47 (s, 1H), 8.29 (bs, 1H), 8.20 (d, J = 9.2 Hz, 1H),

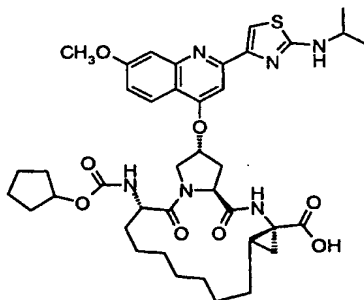
## 97

8.09 (bs, 1H), 7.87 (s, 1H), 7.77 (s, 1H), 7.32 (dd,  $J = 9.2$  Hz, 1H), 7.14 (dd,  $J = 6.7$  Hz, 1H), 5.78 (bs, 1H), 4.58 (dd,  $J = 8.1$  Hz, 2H), 4.43 (bs, 1H), 4.18-4.12 (m, 1H), 3.97 (s, 3H), 3.87 (d,  $J = 8.9$  Hz, 1H), 3.55-3.46 (m, 2H), 2.63-2.53 (m, 1H), 2.47-2.41 (m, 1H), 1.78-1.00 (m, 25H), 1.25 (t,  $J = 7.3$  Hz, 3H). MS;  $m/z$ : 763.1 ( $M + H$ )<sup>+</sup>,  $m/z$ : 761.1 ( $M - H$ )<sup>-</sup>.

## EXAMPLE 35C

## Synthesis of compound #911

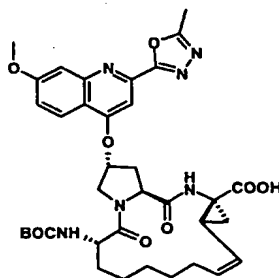
Using the same procedure as described in example 35 but using available *N*-isopropylthiourea gave compound # 911 (Table 9).



<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.47 (s, 1H), 8.29-8.19 (m, 1H), 8.19 (d,  $J = 9.2$  Hz, 1H), 8.09-8.0 (m, 1H), 7.83 (bs, 1H), 7.74 (bs, 1H), 7.31 (d,  $J = 8$  Hz, 1H), 7.14 (d,  $J = 6.4$  Hz, 1H), 5.76 (bs, 1H), 4.64-4.53 (m, 2H), 4.44 (bs, 1H), 4.22-4.09 (m, 3H), 3.97 (s, 3H), 3.87 (d,  $J = 8.6$  Hz, 1H), 2.63-2.58 (m, 1H), 2.46-2.41 (m, 1H), 1.79-1.10 (m, 24H), 1.27 and 1.26 (2 x d,  $J = 6.5$  Hz, 6H). MS;  $m/z$ : 777.0 ( $M + H$ )<sup>+</sup>,  $m/z$ : 775.0 ( $M - H$ )<sup>-</sup>.

## EXAMPLE 36

## Synthesis of compound #716

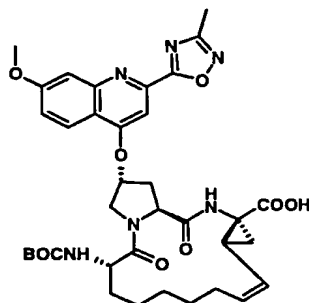


<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  (ppm): 8.62 (s, 1H), 8.13 (d,  $J = 9.2$  Hz, 1H), 7.64-7.54 (m, 2H), 7.47 (d,  $J = 2.6$  Hz, 1H), 7.16 (dd,  $J = 9.2, 2.2$  Hz, 1H), 7.03 (d,  $J = 6.0$  Hz, 1H),

98

5.63 (s, 1H), 5.52 (q, J=9.9 Hz, 1H), 5.26 (t, J=8.9 Hz, 1H), 4.62 (d, J=11.45, 1H), 4.45 (dd, J=9.2, 8.27 Hz, 1H), 4.02 (m, 1H), 3.93 (s, 3H), 3.7 (dd, J=7.6, 1.0 Hz, 1H), 2.66 (s, 3H), 2.55-2.65 (m, 1H), 2.35-2.45 (m, 1H), 2.17 (q, J=8.6 Hz, 1H), 1.65-1.75 (m, 2H), 1.5-1.35 (m, 7H), 1.15 (s, 9H).

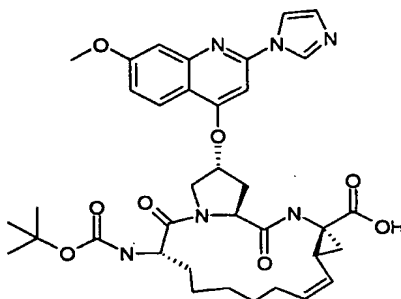
5 MS : 705. (M+1), 703 (M-1)

**EXAMPLE 37****Synthesis of compound #717**

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.62 (s, 1H), 8.15 (d, J=8.9 Hz, 1H), 7.62 (s, 1H), 7.49 (s, 1H), 7.19 (dd, J=9.2, 2.2 Hz, 1H), 7.02 (d, J=5.4 Hz, 1H), 5.64 (s, 1H), 5.52 (q, J=9.9 Hz, 1H), 5.26 (t, J=9.2 Hz, 1H), 4.63 (d, J=11.44, 1H), 4.45 (t, J=9.2 Hz, 1H), 3.94 (s, 3H), 3.9-3.8 (m, 1H), 2.7-2.55 (m, 1H), 2.4-2.3 (m, 1H), 2.18 (q, J=8.9 Hz, 1H), 1.75-1.65 (m, 2H), 1.5-1.2 (m, 7H), 1.14 (s, 9H).

MS : 705. (M+1), 703 (M-1).

15 **EXAMPLE 38**

**Synthesis of compound #718**

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 9.55 (s, 1H), 8.63 (s, 1H), 8.43 (s, 1H), 8.13 (d, J = 9.2 Hz, 1H), 7.66 (s, 1H), 7.46 (s, 1H), 7.32 (d, J = 2.6 Hz, 1H), 7.10-7.07 (m, 2H),

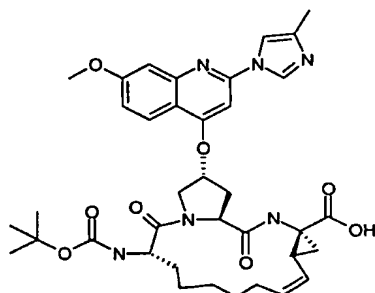
99

5.64-5.54 (m, 1H), 5.59-5.48 (m, 1H), 5.33-5.23 (m, 1H), 4.73-4.61 (m, 1H), 4.45 (dd, J = 7.5, 9.1 Hz, 1H), 4.09-4.00 (m, 1H), 3.92 (s, 3H), 3.93-3.83 (m, 1H), 2.67-2.55 (m, 2H), 2.53-2.43 (m, 1H), 2.42-2.31 (m, 1H), 2.23-2.12 (m, 1H), 1.81-1.66 (m, 2H), 1.52-1.42 (m, 2H), 1.42-1.25 (m, 6H), 1.21 (s, 9H).

5 MS: 689.3 (M+1), 687.3 (M-1)

#### EXAMPLE 39

##### Synthesis of compound #722

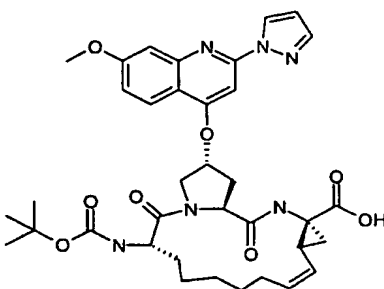


<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 9.70 (s, 1H), 8.64 (s, 1H), 8.26 (s, 1H), 8.14 (d, J = 9.2 Hz, 1H), 7.45 (s, 1H), 7.30 (d, J = 2.5 Hz, 1H), 7.14-7.06 (m, 2H), 5.60-5.54 (m, 1H), 5.58-5.48 (m, 1H), 5.31-5.23 (m, 1H), 4.71-4.62 (m, 1H), 4.49-4.40 (m, 1H), 4.08-3.99 (m, 1H), 3.92 (s, 3H), 3.92-3.84 (m, 1H), 2.69-2.54 (m, 2H), 2.53-2.46 (m, 1H), 2.42-2.31 (m, 1H), 2.37 (s, 3H), 2.22-2.13 (m, 1H), 1.81-1.64 (m, 2H), 1.54-1.42 (m, 2H), 1.42-1.27 (m, 6H), 1.22 (s, 9H).

15 MS: 703.3 (M+1), 701.3 (M-1)

#### EXAMPLE 40

##### Synthesis of compound #733



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.75 (m, 1H), 8.62 (s, 1H), 8.06 (d, J=9.2 Hz, 1H), 7.88-7.87 (m, 1H), 7.48 (s, 1H), 7.28 (d, J=2.6Hz, 1H), 7.05-7.00 (m, 2H), 6.64-6.63

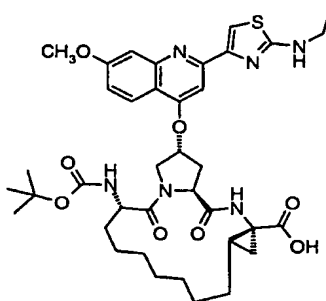
## 100

(m, 1H), 5.62-5.58 (m, 1H), 5.55-5.49 (m, 1H), 5.28-5.24 (m, 1H), 4.64-4.61 (m, 1H), 4.48-4.44 (m, 1H), 4.07-4.03 (m, 1H), 3.91 (s, 3H), 3.92-3.85 (m, 1H), 2.67-2.54 (m, 2H), 2.53-2.45 (m, 1H), 2.41-2.34 (m, 1H), 2.20-2.14 (m, 1H), 1.75-1.69 (m, 2H), 1.50-1.43 (m, 2H), 1.41-1.32 (m, 6H), 1.17 (s, 9H).

5 MS: 689.3 (M+1), 687.2 (M-1)

## EXAMPLE 41

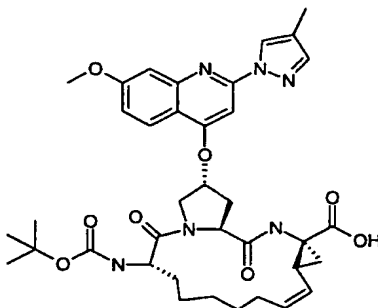
## Synthesis of compound # 703



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 8.50 (s, 1H), 8.19 (s, 1H), 8.17 (s, 1H), 8.11-8.00 (m, 1H), 7.88-7.77 (m, 1H), 7.73 (s, 1H), 7.25 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 6 Hz, 1H), 5.89-5.68 (m, 1H), 4.62 (d, J = 11 Hz, 1H), 4.53 (dd, J = 8.3 Hz, 1H), 4.16-4.07 (m, 1H), 3.96 (s, 3H), 3.88 (bd, J = 9.5 Hz, 1H), 3.53-3.43 (m, 2H), 2.63-2.51 (m, 1H), 2.46-2.36 (m, 1H), 1.81-1.62 (m, 2H), 1.60-1.01 (m, 15H), 1.24 (t, J = 7.4 Hz, 3H), 1.17 (s, 9H).  
MS; es<sup>+</sup>: 751.1(M + H)<sup>+</sup>, es<sup>-</sup>: 749.1- (M - H)<sup>-</sup>.

## 15 EXAMPLE 42

## Synthesis of compound #734



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.62 (s, 1H), 8.54 (s, 1H), 8.04 (d, J=9.2 Hz, 1H), 7.70 (s, 1H), 7.43 (s, 1H), 7.24 (d, J=2.6Hz, 1H), 7.05-6.98 (m, 2H), 5.57-5.54 (m,

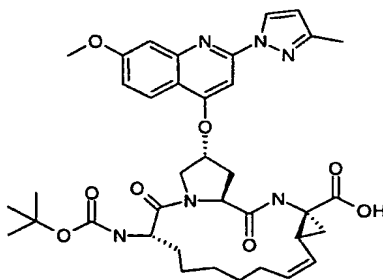
## 101

1H), 5.55-5.48 (m, 1H), 5.28-5.24 (m, 1H), 4.63-4.59 (m, 1H), 4.47-4.43 (m, 1H), 4.13-3.99 (m, 1H), 3.90 (s, 3H), 3.92-3.83 (m, 1H), 2.67-2.55 (m, 2H), 2.53-2.46 (m, 1H), 2.43-2.31 (m, 1H), 2.22-2.15 (m, 1H), 2.15 (3H), 1.75-1.70 (m, 2H), 1.51-1.42 (m, 2H), 1.41-1.28 (m, 6H), 1.17 (s, 9H).

5 MS : 703.2 (M+1), 701.3 (M-1)

## EXAMPLE 43

## Synthesis of compound #738

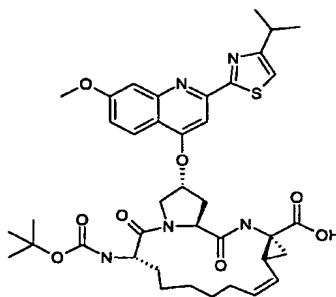


<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.64 (d, J = 2.5 Hz, 1H), 8.62 (s, 1H), 8.04 (d, J = 9.2 Hz, 1H), 7.39 (s, 1H), 7.24 (d, J = 2.5 Hz, 1H), 7.04 (d, J = 6.0 Hz, 1H), 6.99 (dd, J = 2.2, 9.2 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 5.62-5.57 (m, 1H), 5.56-5.47 (m, 1H), 5.31-5.22 (m, 1H), 4.65-4.56 (m, 1H), 4.45 (dd, J = 7.6, 8.9 Hz, 1H), 4.07-4.00 (m, 1H), 3.90 (s, 3H), 3.88-3.84 (m, 1H), 2.68-2.56 (m, 2H), 2.54-2.43 (m, 1H), 2.42-2.31 (m, 1H), 2.34 (s, 3H), 2.24-2.14 (m, 1H), 1.80-1.64 (m, 2H), 1.52-1.43 (m, 2H), 1.43-1.27 (m, 6H), 1.18 (s, 9H).

MS: 703.2 (M+1), 701.2 (M-1)

## EXAMPLE 44

## Synthesis of compound #725



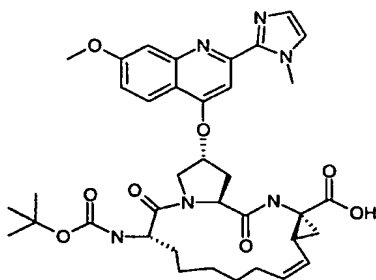
20 <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.62 (s, 1H), 8.10 (d, J=9.2 Hz, 1H), 7.57 (s,

## 102

- 1H), 7.49 (s, 1H), 7.35 (d, J=2.2 Hz, 1H), 7.09-7.03 (m, 2H), 5.65-5.61 (m, 1H), 5.55-5.49 (m, 1H), 5.28-5.24 (m, 1H), 4.62-4.57 (m, 1H), 4.49-4.45 (m, 1H), 4.08-4.01 (m, 1H), 3.93 (s, 3H), 3.92-3.86 (m, 1H), 3.20-3.14 (m, 1H), 2.65-2.56 (s, 1H), 2.53-2.47 (m, 1H) 2.42-2.35 (m, 1H), 2.22-2.15 (m, 1H), 1.79-1.68 (m, 2H), 1.50-1.43 (m, 2H), 1.41-1.28 (m, 12H), 1.18 (s, 9H).
- MS : 748.2 (M+1), 746.2 (M-1)

## EXAMPLE 45

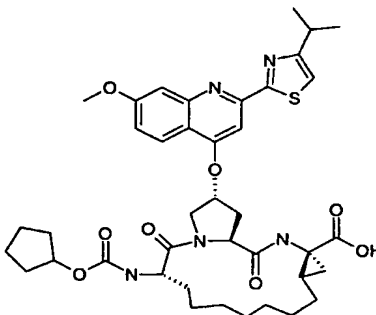
## Synthesis of compound #726



- <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.64 (s, 1H), 8.10 (d, J=9.5 Hz, 1H), 7.83-7.76 (m, 2H), 7.60 (s, 1H), 7.44-7.42 (m, 1H), 7.18-7.01 (m, 2H), 5.56-5.49 (m, 2H), 5.29-5.24 (m, 1H), 4.66-4.63 (m, 1H), 4.47-4.42 (m, 1H), 4.28 (s, 3H), 4.06-4.02 (m, 2H), 3.94 (s, 3H), 3.93-3.86 (m, 1H), 2.66-2.55 (m, 2H), 2.42-2.31 (m, 2H), 2.22-2.14 (m, 1H), 1.79-1.65 (m, 2H), 1.52-1.27 (m, 7H), 1.22 (s, 9H).
- MS : 703.2 (M+1), 701.3 (M-1)

## EXAMPLE 46

## Synthesis of compound #906



- <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.46 (s, 1H), 8.06 (d, J=9.2 Hz, 1H), 7.57 (s, 1H), 7.49 (s, 1H), 7.34 (m, 1H), 7.14-7.05 (m, 2H), 5.63-5.58 (m, 1H), 4.66-4.61 (m, 1H),



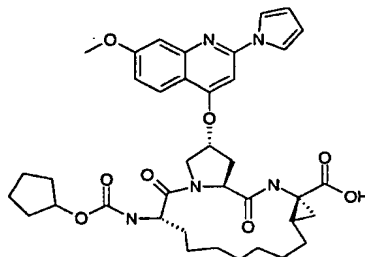
## 103

4.54-4.44 (m, 2H), 4.23-4.18 (m, 1H) 3.93 (s, 3H), 3.92-3.88 (m, 1H), 3.21-3.14 (m, 1H),  
2.44-2.33 (m, 1H), 1.35 (d, J=7Hz, 6H), 1.73-1.01 (m, 26H)

MS : 762.0 (M+1), 759.9 (M-1)

## EXAMPLE 47

## 5 Synthesis of compound #907



<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ (ppm): 8.46 (s, 1H), 7.98 (d, J=8.9 Hz, 1H), 7.91-7.89  
(m, 2H), 7.23-7.21 (m, 2H), 7.07-7.00 (m, 2H), 6.35-6.32 (m, 2H), 5.64-5.58 (m, 1H),  
4.65-4.61 (m, 1H), 4.53-4.47 (m, 2H), 4.24-4.19 (m, 1H) 3.90 (s, 3H), 3.86-3.84 (m, 1H),  
10 2.40-2.33 (m, 1H), 1.73-1.01 (m, 26H).

MS : 702.0 (M+1), 699.9 (M-1)

## EXAMPLE 48

## Full-length NS3-NS4A heterodimer protein fluorogenic assay

The NS2-NS5B-3' non coding region was cloned by RT-PCR into the pCR®3 vector  
15 (Invitrogen) using RNA extracted from the serum of an HCV genotype 1b infected  
individual (provided by Dr. Bernard Willems, Hôpital St-Luc, Montréal, Québec,  
Canada). The NS3-NS4A region (NS3-NS4AFL) was then subcloned by PCR into the  
pFastBac™ HTa baculovirus expression vector (Gibco/BRL). The vector sequence  
includes a region encoding a 28-residue N-terminal sequence which contains a  
20 hexahistidine tag. The Bac-to-Bac™ baculovirus expression system (Gibco/BRL) was  
used to produce the recombinant baculovirus. His-NS3-NS4AFL was expressed by  
infecting 10<sup>6</sup> Sf21 cells/mL with the recombinant baculovirus at a multiplicity of  
infection of 0.1-0.2 at 27°. Authentic auto-proteolysis occurs during expression to  
produce a non covalent and stable NS3-NS4A protein complex (referred to as full-  
25 length "FL"). The infected culture was harvested 48 to 64 h later by centrifugation at  
4°. The cell pellet was homogenized in 50mM NaPO<sub>4</sub>, pH 7.5, 40% glycerol (w/v),

2mM  $\beta$ -mercaptoethanol, in presence of a cocktail of protease inhibitors. His-NS3-NS4AFL was then extracted from the cell lysate with 1.5% NP-40, 0.5% Triton X-100, 0.5M NaCl, and a DNase treatment. After ultracentrifugation, the soluble extract was diluted 4-fold and bound on a Pharmacia Hi-Trap Ni-chelating column. The

5 His-NS3-NS4AFL was eluted in a >90% pure form (as judged by SDS-PAGE), using a 50 to 400 mM imidazole gradient. The His-NS3-NS4AFL was stored at -80° in 50 mM sodium phosphate, pH 7.5, 10% (w/v) glycerol, 0.5 M NaCl, 0.25 M imidazole, 0.1% NP-40. It was thawed on ice and diluted just prior to use.

The protease activity of His-NS3-NS4AFL was assayed in 50 mM Tris-HCl, pH 8.0,

10 0.25 M sodium citrate, 0.01% (w/v) n-dodecyl- $\beta$ -D-maltoside, 1 mM TCEP. Five (5)  $\mu$ M of the internally quenched substrate anthranilyl-DDIVPAbu[C(O)-O]-AMY(3-NO<sub>2</sub>)TW-OH in presence of various concentrations of inhibitor were incubated with 1.5 nM of His-NS3-NS4AFL for 45 min at 23°. The final DMSO concentration did not exceed 5.25%. The reaction was terminated with the addition of 1M MES, pH 5.8.

15 Fluorescence of the N-terminal product was monitored on a Perkin-Elmer LS-50B fluorometer equipped with a 96-well plate reader (excitation wavelength: 325 nm; emission wavelength: 423 nm).

The % inhibition was calculated with the following equation:

$$100 - [(flu_{inh} - flu_{blank}) / (flu_{ctl} - flu_{blank}) \times 100]$$

20 A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC<sub>50</sub>) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc. Cary, N.C.).

#### EXAMPLE 49

##### Recombinant HCV NS3 protease radiometric assay

25 The substrate used for the HCV NS3 protease radiometric assay, DDIVPC-SMSYTW, is cleaved between the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW and the tracer biotin-DDIVPC-SMS[<sup>125</sup>I-Y]TW were incubated

30 with the recombinant NS3 protease in the absence or in the presence of inhibitors. The separation of substrate from products was performed by adding avidin-coated

agarose beads to the assay mixture followed by filtration. The amount of SMS[<sup>125</sup>I-Y]TW product found in the filtrate (with or without inhibitor) allowed for the calculation of the percentage of substrate conversion and of the percentage of inhibition.

5 A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from Life Technologies. Glycerol (UltraPure), MES and BSA were purchased from Sigma<sup>®</sup>. TCEP was obtained from Pierce, DMSO from Aldrich<sup>®</sup> and NaOH from Anachemia<sup>®</sup>.

Assay buffer: 50 mM Tris-HCl, pH 7.5, 30% (w/v) glycerol, 2% (w/v) CHAPS, 1  
10 mg/mL BSA, 1 mM TCEP (TCEP added just prior to use from a 1 M stock solution in water).

Substrate: DDIVPC-SMSYTW, 25  $\mu$ M final concentration (from a 2 mM stock solution in DMSO stored at -20°C to avoid oxidation).

Tracer: reduced mono-iodinated substrate(biotin-DDIVPC-SMS[<sup>125</sup>I-Y]TW) ( $\approx$  1 nM  
15 final concentration).

HCV NS3 protease type 1b, 25 nM final concentration (from a stock solution in 50 mM sodium phosphate, pH 7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-40).

B. Protocol

20 The assay was performed in a 96-well polypropylene plate. Each well contained:  
20  $\mu$ L substrate/tracer in assay buffer;

10  $\mu$ L  $\pm$  inhibitor in 20% DMSO/assay buffer;

10  $\mu$ L NS3 protease 1b.

Blank (no inhibitor and no enzyme) and control (no inhibitor) were also prepared on  
25 the same assay plate.

The enzymatic reaction was initiated by the addition of the enzyme solution and the assay mixture was incubated for 60 min at 23°C under gentle agitation. Twenty (20)  $\mu$ L of 0.025 N NaOH were added to quench the enzymatic reaction.

Twenty (20)  $\mu$ L of avidin-coated agarose beads (purchased from Pierce<sup>®</sup>) were  
30 added in a Millipore<sup>®</sup> MADP N65 filtration plate. The quenched assay mixture was transferred to the filtration plate, and incubated for 60 min at 23°C under gentle agitation.

The plates were filtered using a Millipore® MultiScreen Vacuum Manifold Filtration apparatus, and 40 µL of the filtrate was transferred to an opaque 96-well plate containing 60 µL of scintillation fluid per well.

The filtrates were counted on a Packard® TopCount instrument using a <sup>125</sup>I-liquid  
5 protocol for 1 minute.

The %inhibition was calculated with the following equation:

$$100 - [(counts_{inh} - counts_{blank}) / (counts_{ctrl} - counts_{blank}) \times 100]$$

A non-linear curve fit with the Hill model was applied to the inhibition-  
concentration data, and the 50% effective concentration (IC<sub>50</sub>) was calculated by the  
10 use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

#### EXAMPLE 50

##### Specificity assays

The specificity of the compounds was determined against a variety of serine  
proteases: human leukocyte elastase, porcine pancreatic elastase and bovine  
15 pancreatic α-chymotrypsin and one cysteine protease: human liver cathepsin B. In  
all cases a 96-well plate format protocol using a chromogenic substrate specific for  
each enzyme was used. Each assay included a 1 h enzyme-inhibitor pre-incubation  
at RT followed by addition of substrate and hydrolysis to ≈30% conversion as  
measured on a UV Thermomax® microplate reader or a fluorescence Perkin-Elmer®  
20 LS50B plate reader. Substrate concentrations were kept as low as possible compared  
to K<sub>M</sub> to reduce substrate competition. Compound concentrations varied from 300  
to 0.06 µM depending on their potency.

The final conditions for each assay were as follows:

50mM Tris-HCl pH 8, 0.5 M Na<sub>2</sub>SO<sub>4</sub>, 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01%  
25 Tween-20 with;

[100 µM Succ-AAPF-pNA and 250 pM α-chymotrypsin], [133 µM Succ-AAA-pNA  
and 8 nM porcine elastase], [133 µM Succ-AAV-pNA and 8 nM leukocyte elastase];  
or

[100 mM NaHPO<sub>4</sub> pH 6, 1 mM EDTA, 3% DMSO, 1mM TCEP, 0.01% Tween-20, 4  
30 µM Z-FR-AMC (7-amino-4-methylcoumarin) and 0.5 nM cathepsin B (the stock  
enzyme was activated in buffer containing 20 mM TCEP before use)].

A representative example is summarized below for porcine pancreatic elastase:

In a polystyrene flat-bottom 96-well plate (Cellwells, Corning) were added using a Biomek liquid handler (Beckman):

40  $\mu$ L of assay buffer (50 mM Tris-HCl pH 8, 1 M Na<sub>2</sub>SO<sub>4</sub>, 50 mM NaCl, 0.1 mM EDTA);

20  $\mu$ L of enzyme solution (50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 40 nM porcine pancreatic elastase); and

20  $\mu$ L of inhibitor solution (50 mM Tris-HCl, pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 1.5 mM-0.3  $\mu$ M inhibitor, 15% v/v DMSO).

After 60 min pre-incubation at RT, 20  $\mu$ L of substrate solution (50 mM Tris-HCl, pH 8, 0.5 M Na<sub>2</sub>SO<sub>4</sub>, 50 mM NaCl, 0.1 mM EDTA, 665  $\mu$ M Succ-AAA-pNA) were added to each well and the reaction was further incubated at RT for 60 min after which time the absorbance was read on the UV Thermomax® plate reader. Rows of wells were allocated for controls (no inhibitor) and for blanks (no inhibitor and no enzyme).

The sequential 2-fold dilutions of the inhibitor solution were performed on a separate plate by the liquid handler using 50 mM Tris-HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 15% (v/v) DMSO. All other specificity assays were performed in a similar fashion.

The percentage of inhibition was calculated using the formula:

$$[1 - ((UV_{inh} - UV_{blank}) / (UV_{ctl} - UV_{blank}))] \times 100$$

A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (IC<sub>50</sub>) was calculated by the use of SAS software (Statistical Software System; SAS Institute, Inc., Cary, N.C.).

## EXAMPLE 51

### NS3 Protease Cell-based assay

This assay is done with Huh-7 cells, a human cell line derived from a hepatoma, co-transfected with 2 DNA constructs:

one (called NS3) expressing part of the HCV non-structural polyprotein fused to the tTA protein through an NS5A-NS5B cleavage site in the following order: NS3-NS4A-NS4B-NS5A-(NS5B)tTA where (NS5B) represents the 6 first amino acids of NS5B.

This polyprotein is expressed under the control of the CMV promoter, the other (called SEAP) expressing the reporter protein, secreted alkaline phosphatase (SEAP), under the regulation of a tTA-responsive promoter.

The first construct leads to the expression of a polyprotein from which the different  
5 mature proteins are released through cleavage by the NS3 protease. It is believed that the mature viral proteins forms a complex at the membrane of the endoplasmic reticulum. tTA is a fusion protein, described by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992): 5547-5551), which contains a DNA-binding domain and a transcriptional activator. Release of the tTA protein requires an NS3-dependent  
10 cleavage at the NS5A-NS5B cleavage site between NS5A and itself. This last cleavage allows tTA to migrate to the nucleus and transactivate the SEAP gene. Therefore, reduction of NS3 proteolytic activity leads to confinement of tTA to the cytoplasm and concomitant decrease in SEAP activity.

To control for cellular activities other than inhibition of NS3 protease which are due  
15 to the compound, a parallel co-transfection is done with a construct expressing tTA alone and the same reporter construct such that SEAP activity is independent of the NS3 protease.

**Protocol of the assay:** Huh-7 cells, grown in CHO-SFMII (Life Technologies) + 10% FCS (fetal calf serum) were co-transfected with the two DNA constructs in the  
20 following proportions:

7 µg NS3+ 500ng SEAP + 800µl FuGENE (Boehringer Mannheim) per 4 X 10<sup>6</sup> Huh-7 cells. After 5 hours at 37°C, the cells were washed, trypsinized and plated (at 80 000 cells/well) in 96-well plates containing a range of concentrations of the compounds to be tested. After a 24-hour incubation period, the SEAP activity in the medium was  
25 measured with the Phospha-Light kit (Tropix).

Analysis of the percent inhibition of SEAP activity with respect to compound concentration was performed with the SAS software to obtain the EC<sub>50</sub>.

## TABLES OF COMPOUNDS

The following tables list compounds representative of the invention.

All compounds listed in Tables 1 to 9 were found to be active in the enzymatic assay presented in Example 48. A number accompanied by an asterisk (\*) represents

5 enzymatic activity obtained with the radiometric assay presented in Example 49 with  $IC_{50}$ 's under  $50\mu M$ . In these enzymatic assays, the following grading was used:

$A \geq 1\mu M$ ;  $1\mu M > B > 0.1\mu M$ ; and  $C \leq 0.1\mu M$ .

Several compounds were tested in the specificity assays presented in Example 50 and were found to be specific for the NS3 protease. In general, the results from the

10 different specificity assays are the following: HLE  $>300\mu M$ ; PPE  $>300\mu M$ ;  $\alpha$ -Chym.  $>300\mu M$ ; Cat. B  $>300\mu M$ ; indicating that these compounds are highly specific toward the HCV NS3 protease and are not expected to exhibit serious side-effects.

In addition, certain of these compounds were tested in the cell-based assay

described in Example 51 and were found to have activity with an  $EC_{50}$  below  $10\mu M$ ,

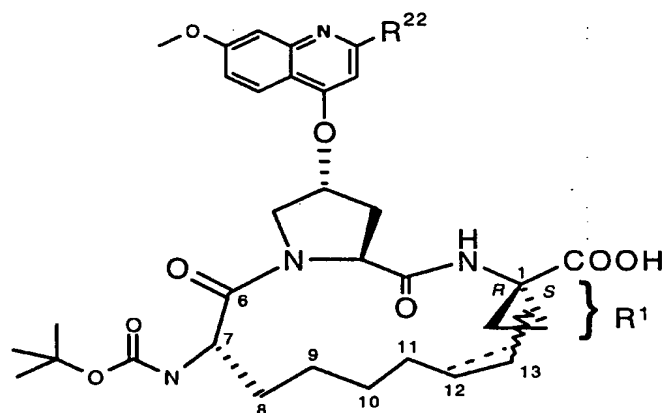
15 strongly indicating that these compounds can cross the cell membrane. Particularly, compounds of Tables 7, 8 and 9 have been assessed in the cellular assay and the result indicated in the last column. In this cellular assay, the following coding was used: **A**  $>1\mu M$ ; **B**  $\leq 1\mu M$ .

The following abbreviations are used within the following tables:

20 MS: Electrospray mass spectral data;  $m/z$   $MH^+$  except when otherwise indicated by an asterisk\* =  $m/z$   $MH^-$ ; Ac: acetyl; Bn: benzyl; Boc: *tert*-butyloxycarbonyl; Ph: phenyl; Pr: propyl.

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TABLE 1

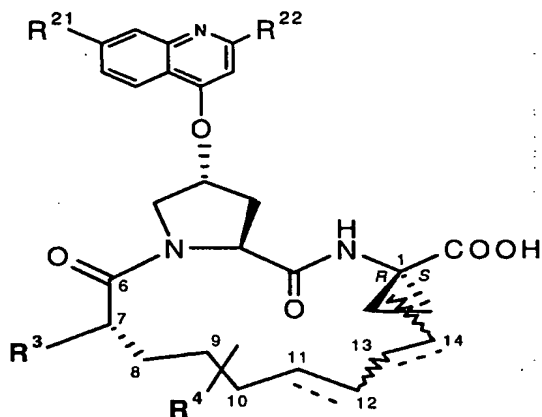
single stereoisomer at  $R^1$ 

| Cpd. # | double bond         | D- $R^1$ bond stereochem  | $R^{22}$ | MS    | enzyme activity. |
|--------|---------------------|---------------------------|----------|-------|------------------|
| 101    | 12,13- <i>trans</i> | 1R, D <i>syn</i> to amide | phenyl   | 685.8 | A*               |
| 102    | none                | 1R, D <i>syn</i> to acid  | phenyl   | 687.2 | C                |
| 103    | none                | 1R D <i>syn</i> to amide  | phenyl   | 687.2 | A*               |




111

TABLE 2

single stereoisomer at R<sup>1</sup>

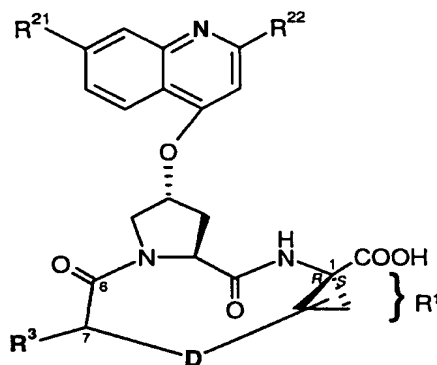
| Cpd # | R <sup>3</sup>              | R <sup>4</sup>        | double bond         | D-R <sup>1</sup> bond stereochem | R <sup>21</sup> | R <sup>22</sup> | MS    | enz. act. |
|-------|-----------------------------|-----------------------|---------------------|----------------------------------|-----------------|-----------------|-------|-----------|
| 202   | NH-Boc                      | H                     | 11,12- <i>trans</i> | 1R or 1S, D syn to acid          | H               | H               | 593.7 | B         |
| 203   | NH-acetyl                   | H                     | 11,12- <i>trans</i> | 1R or 1S, D syn to acid          | H               | H               | 535.6 | A         |
| 205   | NH-Boc                      | 11-OH<br>12-OH<br>cis | none                | 1R or 1S, D syn to acid          | H               | H               | 627.7 | B         |
| 206   | NH-Boc                      | H                     | 13,14- <i>cis</i>   | 1R, D syn to acid                | H               | H               | 593.7 | C         |
| 207   | NH-Boc                      | H                     | 13,14- <i>cis</i>   | 1R, D syn to acid                | OMe             | H               | 623.7 | C         |
| 208   | NH-Boc                      | H                     | 13,14- <i>cis</i>   | 1R, D syn to acid                | OMe             | phenyl          | 699.8 | C         |
| 209   | NH-C(O)-<br>NH- <i>t</i> Bu | H                     | 13,14- <i>cis</i>   | 1R, D syn to acid                | OMe             | phenyl          | 698.8 | C         |

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| Cpd # | R <sup>3</sup>     | R <sup>4</sup>             | double bond       | D-R <sup>1</sup> bond stereochem | R <sup>21</sup> | R <sup>22</sup>   | MS    | enz. act. |
|-------|--------------------|----------------------------|-------------------|----------------------------------|-----------------|---|-------|-----------|
| 210   | NH-Boc             | H                          | 13,14- <i>cis</i> | 1S, D syn to acid                | OMe             | phenyl  | 699.8 | A*        |
| 211   | NH <sub>2</sub>    | H                          | 13,14- <i>cis</i> | 1R, D syn to acid                | OMe             | phenyl  | 599.7 | C         |
| 213   | OH<br>(one isomer) | H                          | 13,14- <i>cis</i> | 1R, D syn to acid                | OMe             | H   | 524.6 | B         |
| 214   | NH-Boc             | 10-oxo                     | 13,14- <i>cis</i> | 1R, D syn to acid                | OMe             | phenyl  | 713.8 | C         |
| 215   | NH-Boc             | H                          | none              | 1R, D syn to acid                | OMe             | phenyl  | 701.8 | C         |
| 217   | NH-Boc             | 10-OH<br>(mixt dia stereo) | 13,14- <i>cis</i> | 1R, D syn to acid                | OMe             | phenyl  | 715.8 | C         |
| 218   | NH-Boc             | 10-oxo                     | 13,14- <i>cis</i> | 1R, D <i>syn</i> to amide        | OMe             | phenyl  | 713.8 | C         |
| 219   | NH-Ac              | H                          | none              | 1R, D syn to acid                | OMe             | phenyl  | 643.2 | C         |
| 220   | NH-Boc             | H                          | 13,14- <i>cis</i> | 1R, D <i>syn</i> to amide        | OMe             |  | 706.2 | C         |

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TABLE 3

single stereoisomer at R<sup>1</sup>

| Cpd.<br># | R <sup>3</sup> | - D - | D-R <sup>1</sup> bond<br>stereochem | R <sup>21</sup> | R <sup>22</sup> | MS    | enz<br>act. |
|-----------|----------------|-------|-------------------------------------|-----------------|-----------------|-------|-------------|
| 301       | NH-<br>Boc     |       | 1R or 1S,<br>D syn to<br>acid       | H               | H               | 621.7 | B           |
| 302       | NH-<br>Boc     |       | 1R, D <i>syn</i><br>to amide        | OMe             | Ph              | 671   | A           |
| 303       | NH-<br>Boc     |       | 1R, D <i>syn</i><br>to amide        | OMe             | Ph              | 701.3 | B           |
| 304       | NH-<br>Boc     |       | 1R, D <i>syn</i><br>to acid         | OMe             | Ph              | 711.1 | C           |
| 305       | HO             |       | 1R, D <i>syn</i><br>to acid         | OMe             | Ph              | 602.2 | B           |
| 306       | NH-<br>Boc     |       | 1R, D <i>syn</i><br>to amide        | OMe             | Ph              | 673.2 | A*          |

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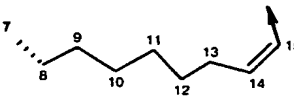
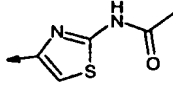
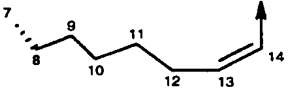
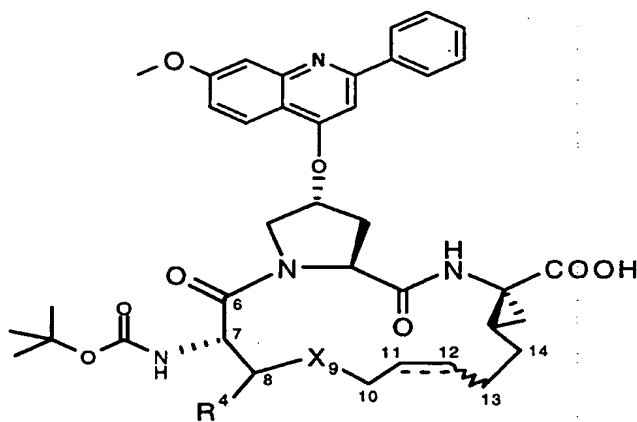







| Cpd.<br># | R <sup>3</sup> | - D -   | D-R <sup>1</sup> bond<br>stereochem | R <sup>21</sup> | R <sup>22</sup>   | MS    | enz<br>act. |
|-----------|----------------|---|-------------------------------------|-----------------|---|-------|-------------|
| 307       | NH-Boc         |  | 1R, D <i>syn</i><br>to acid         | OMe             |  | 777   | C           |
| 308       | NH-Ac          |  | 1R, D <i>syn</i><br>to acid         | OMe             | OEt   | 609.2 | C           |

TABLE 4

D-R<sup>1</sup> bond *syn* to the acid

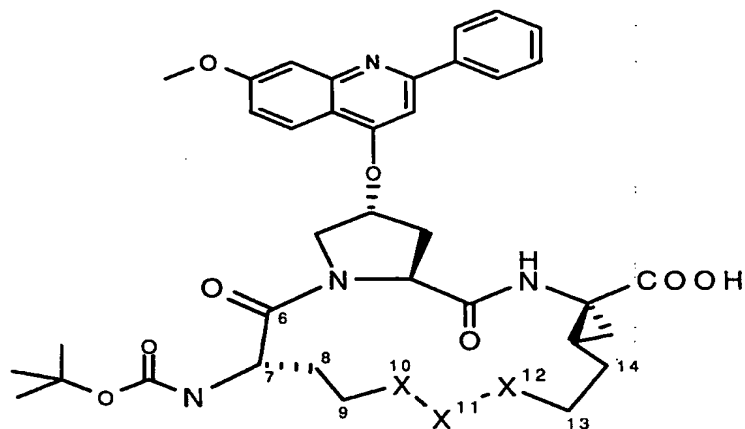
| Cpd<br># | R <sup>4</sup>  | 9-X             | 11, 12<br>double<br>bond | MS    | enzyme<br>activity. |
|----------|---|-----------------|--------------------------|-------|---------------------|
| 401      | H   | CH <sub>2</sub> | trans                    | 699.3 | C                   |
| 402      | H   | CH <sub>2</sub> | cis                      | 699.4 | B                   |
| 403      | H   | O               | trans                    | 701.3 | C                   |
| 404      |  | O               | trans                    | 715.3 | B                   |

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| Cpd # | R <sup>4</sup>   | 9-X | 11, 12 double bond | MS    | enzyme activity. |
|-------|--|-----|--------------------|-------|------------------|
| 405   | Me    | O   | trans              | 715.2 | C                |
| 406   | H  | O   | none               | 703.3 | C                |
| 407   | Me    | O   | none               | 717.3 | B                |
| 408   | Me    | O   | none               | 717.3 | C                |
| 409   | Me    | O   | cis                | 715.2 | B                |
| 410   | Me    | S   | trans              | 731.3 | C                |
| 411   | Me  | S   | cis                | 731.3 | A*               |
| 412   | 8-(Me) <sub>2</sub>  | 9-S | cis                | 745.3 | A                |

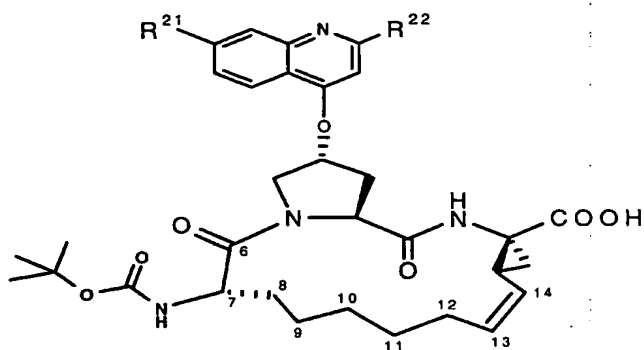
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TABLE 5

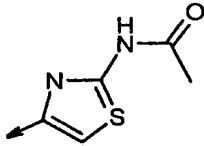
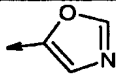
D-R<sup>1</sup> bond *syn* to the acid

| Cpd # | 10-X            | 11-X            | 12-X            | MS    | enzyme activity. |
|-------|-----------------|-----------------|-----------------|-------|------------------|
| 501   | CH <sub>2</sub> | O               | CH <sub>2</sub> | 703.2 | C                |
| 502   | CH <sub>2</sub> | CH <sub>2</sub> | CH <sub>2</sub> | 701   | C                |
| 503   | CH <sub>2</sub> | CH <sub>2</sub> | NH              | 702.3 | A                |
| 504   | CH <sub>2</sub> | CH <sub>2</sub> | N(Me)           | 716.3 | A*               |
| 505   | CH <sub>2</sub> | CH <sub>2</sub> | N(CO)Me         | 744.3 | B                |
| 506   | CH <sub>2</sub> | CH <sub>2</sub> | N(CO)Ph         | 806.3 | B                |
| 507   | NH              | CH <sub>2</sub> | CH <sub>2</sub> | 702.3 | C                |
| 508   | N(CO)Me         | CH <sub>2</sub> | CH <sub>2</sub> | 744.3 | C                |

### TABLE 6

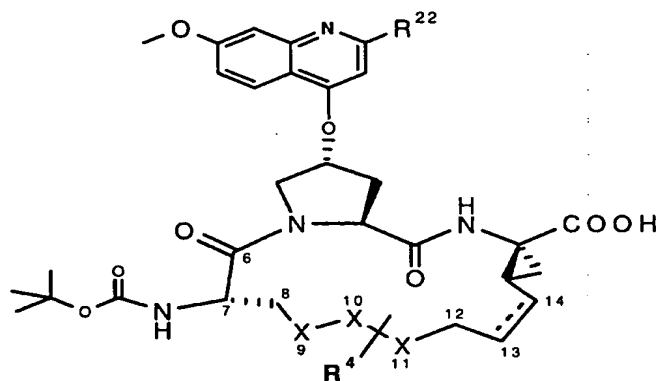


**D-R<sup>1</sup> bond *syn* to the acid**

| Cpd # | R <sup>21</sup>    | R <sup>22</sup>   | MS     | enzyme activity. |
|-------|--------------------|---|--------|------------------|
| 601   | N(Me) <sub>2</sub> |   | 776.2  | C                |
| 602   | OH                 | (CF <sub>3</sub> )  | 675.2* | C                |
| 603   | OMe                |  | 690.1  | C                |

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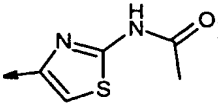
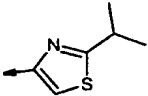
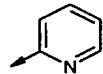
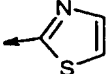

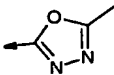
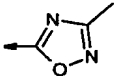

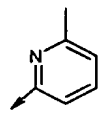
TABLE 7

D-R' bond *syn* to the acid

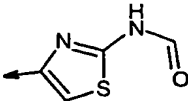
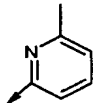
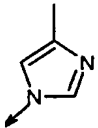

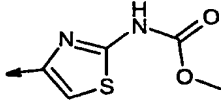
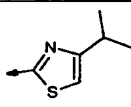

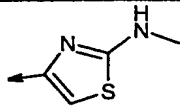
| Cpd # | R <sup>4</sup> | X <sup>9</sup> ; X <sup>10</sup> ; or<br>X <sup>11</sup> | 13,14<br>double<br>bond | R <sup>22</sup> | MS    | cell.<br>act. |
|-------|----------------|--|-------------------------|-----------------|-------|---------------|
| 701   | H              | 11-O   | Cis                     | phenyl          | 701.3 | A             |
| 702   | H              | CH <sub>2</sub>  | Cis                     |                 | 763.1 | B             |
| 703   | H              | CH <sub>2</sub>  | None                    |                 | 751.4 | B             |
| 704   | H              | CH <sub>2</sub>  | Cis                     |                 | 700.3 | B             |
| 705   | H              | CH <sub>2</sub>  | Cis                     |                 | 706.2 | B             |
| 707   | H              | CH <sub>2</sub>  | Cis                     |                 | 748.2 | B             |
| 708   | H              | CH <sub>2</sub>  | Cis                     |                 | 749.2 | B             |



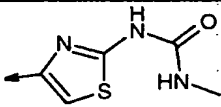
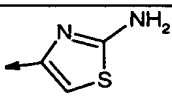


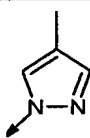
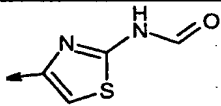
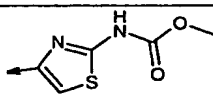
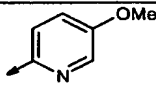
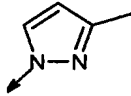
119

| Cpd # | R <sup>4</sup> | X <sup>9</sup> ; X <sup>10</sup> ; or<br>X <sup>11</sup> | 13,14<br>double<br>bond | R <sup>22</sup>   | MS    | cell.<br>act. |
|-------|----------------|--|-------------------------|---|-------|---------------|
| 709   | H              | CH <sub>2</sub>  | None                    |   | 765.2 | B             |
| 710   | H              | CH <sub>2</sub>  | None                    |   | 750.1 | B             |
| 711   | H              | CH <sub>2</sub>  | None                    |    | 702.2 | B             |
| 712   | H              | CH <sub>2</sub>  | Cis                     | -OEt  | 667.3 | B             |
| 713   | H              | CH <sub>2</sub>  | None                    |    | 708.1 | B             |
| 714   | H              | CH <sub>2</sub>  | None                    | -OEt  | 669.3 | B             |
| 715   | H              | CH <sub>2</sub>  | Cis                     |  | 688.3 | B             |
| 716   | H              | CH <sub>2</sub>  | Cis                     |  | 705.3 | A             |
| 717   | H              | CH <sub>2</sub>  | Cis                     |  | 705.2 | B             |
| 718   | H              | CH <sub>2</sub>  | Cis                     |  | 689.3 | B             |
| 719   | H              | CH <sub>2</sub>  | Cis                     |  | 714.2 | B             |

120

| Cpd # | R <sup>4</sup> | X <sup>9</sup> ; X <sup>10</sup> ; or<br>X <sup>11</sup> | 13,14<br>double<br>bond | R <sup>22</sup>  | MS    | cell.<br>act. |
|-------|----------------|--|-------------------------|--|-------|---------------|
| 720   | H              | CH <sub>2</sub>  | None                    |    | 751.2 | B             |
| 721   | H              | CH <sub>2</sub>  | None                    |     | 716.3 | B             |
| 722   | H              | CH <sub>2</sub>  | Cis                     |     | 703.3 | B             |
| 723   | H              | CH <sub>2</sub>  | None                    |     | 690.3 | B             |
| 724   | H              | CH <sub>2</sub>  | None                    |  | 781.1 | B             |
| 725   | H              | CH <sub>2</sub>  | Cis                     |  | 748.2 | B             |
| 726   | H              | CH <sub>2</sub>  | Cis                     |   | 703.2 | B             |
| 727   | H              | CH <sub>2</sub>  | Cis                     | -CH <sub>2</sub> -OMe  | 667.3 | A             |
| 728   | H              | CH <sub>2</sub>  | Cis                     | Me   | 637.3 | A             |
| 729   | H              | CH <sub>2</sub>  | Cis                     |  | 735.2 | B             |

121

| Cpd # | R <sup>4</sup> | X <sup>9</sup> ; X <sup>10</sup> ; or<br>X <sup>11</sup> | 13,14<br>double<br>bond | R <sup>22</sup>  | MS    | cell.<br>act. |
|-------|----------------|--|-------------------------|--|-------|---------------|
| 730   | H              | CH <sub>2</sub>  | None                    |    | 780.1 | B             |
| 731   | H              | CH <sub>2</sub>  | Cis                     |    | 721.1 | B             |
| 732   | H              | CH <sub>2</sub>  | Cis                     |     | 705.3 | A             |
| 733   | H              | CH <sub>2</sub>  | Cis                     |     | 689.3 | B             |
| 734   | H              | CH <sub>2</sub>  | Cis                     |    | 703.2 | B             |
| 735   | H              | CH <sub>2</sub>  | Cis                     |  | 749.2 | B             |
| 736   | H              | CH <sub>2</sub>  | Cis                     |  | 779.2 | B             |
| 737   | H              | CH <sub>2</sub>  | Cis                     |  | 730.2 | B             |
| 738   | H              | CH <sub>2</sub>  | Cis                     |  | 703.2 | B             |
| 739   | 10-(R)<br>Me   | CH <sub>2</sub>  | None                    | Ph   | 715.2 | B             |
| 740   | 10-(S)<br>Me   | CH <sub>2</sub>  | none                    | Ph   | 715.3 | B             |

122

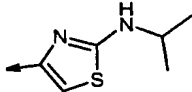
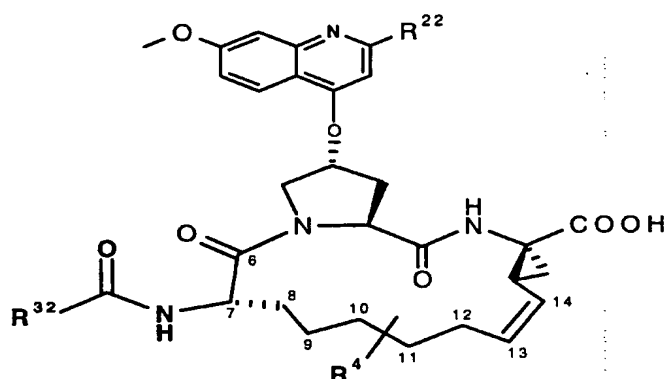
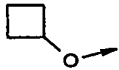
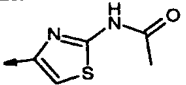
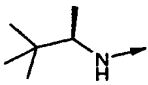
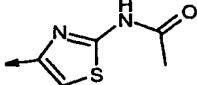
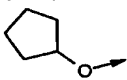
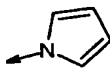
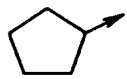
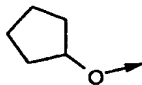
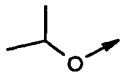
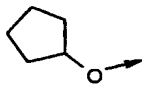
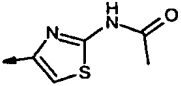
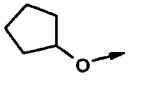
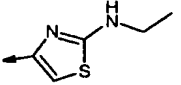
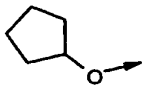
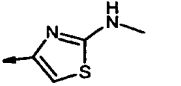
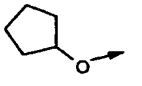
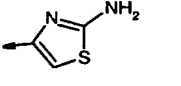

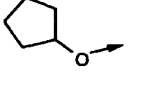
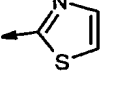
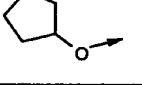
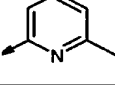
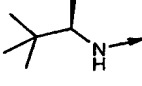
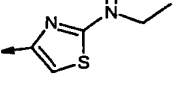
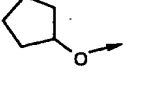
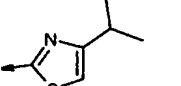
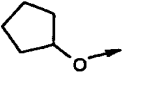
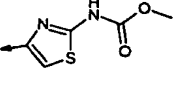
| Cpd # | R <sup>4</sup> | X <sup>9</sup> ; X <sup>10</sup> ; or<br>X <sup>11</sup> | 13,14<br>double<br>bond | R <sup>22</sup>  | MS    | cell.<br>act. |
|-------|----------------|--|-------------------------|--|-------|---------------|
| 741   | H              | CH <sub>2</sub>  | Cis                     |  | 763.1 | B             |

TABLE 8

D-R<sup>1</sup> bond *syn* to the acid, double bond 13,14: *cis*

| Cpd # | R <sup>32</sup>   | R <sup>4</sup> | R <sup>22</sup>  | MS    | cell.<br>act. |
|-------|---|----------------|--|-------|---------------|
| 801   |  | H              |  | 761.2 | B             |
| 803   | <i>n</i> -Pr  | H              | OEt  | 637.3 | A             |
| 804   |  | H              |  | 790.3 | B             |
| 805   |  | H              |   | 700.1 | B             |
| 806   |  | H              | OEt  | 663.2 | A             |

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| Cpd # | R <sup>32</sup>   | R <sup>4</sup> | R <sup>22</sup>  | MS    | cell.<br>act. |
|-------|---|----------------|--|-------|---------------|
| 807   |    | H              | OEt  | 679.3 | B             |
| 808   |    | H              | OEt  | 653.2 | A             |
| 809   |    | H              |    | 775.1 | B             |
| 810   |    | H              |    | 761.2 | B             |
| 811   |    | H              |    | 747.2 | B             |
| 812   |   | H              |   | 733.2 | B             |
| 813   |  | H              | OEt  | 691.3 | B             |
| 814   |  | H              |   | 718.3 | B             |
| 815   |  | H              |   | 726.3 | B             |
| 816   |  | H              |  | 776.3 | B             |
| 817   |  | H              |  | 760.2 | B             |
| 818   |  | H              |  | 791.1 | B             |

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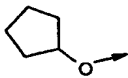
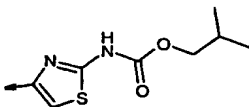
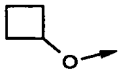
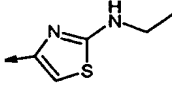
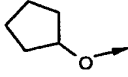

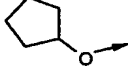
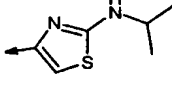
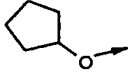

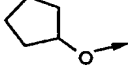
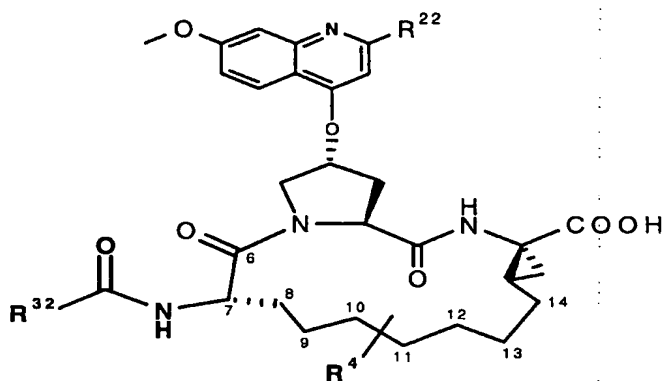
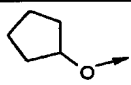
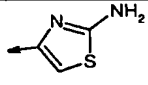
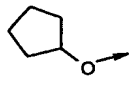
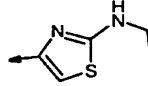
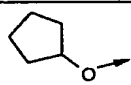
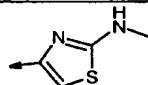
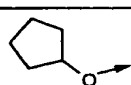
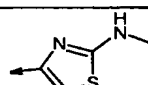

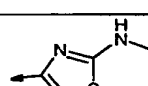
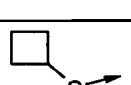
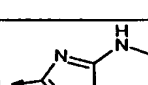
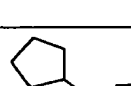
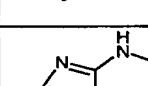
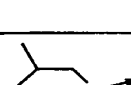
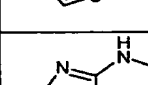
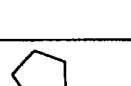
| Cpd # | R <sup>32</sup>  | R <sup>4</sup> | R <sup>22</sup>  | MS    | cell.<br>act. |
|-------|--|----------------|--|-------|---------------|
| 819   |   | H              |  | 833.2 | B             |
| 820   |   | H              |  | 747.2 | B             |
| 821   |   | H              |   | 700.9 | B             |
| 822   |   | H              |  | 775.4 | B             |
| 823   |   | H              |   | 715.2 | B             |
| 824   |  | 10- (R)<br>Me  | OEt  | 693.0 | B             |

TABLE 9

D-R<sup>1</sup> bond *syn* to the acid

| Cpd # | R <sup>32</sup> | R <sup>4</sup> | R <sup>22</sup> | MS    | cell. act. |
|-------|-----------------|----------------|-----------------|-------|------------|
| 901   |                 | H              | OEt             | 681.3 | B          |
| 902   |                 | H              |                 | 719.9 | B          |
| 903   |                 | H              |                 | 705.9 | B          |
| 904   |                 | H              |                 | 703.0 | B          |
| 905   |                 | H              |                 | 689.0 | B          |
| 906   |                 | H              |                 | 762.0 | B          |
| 907   |                 | H              |                 | 702.0 | B          |

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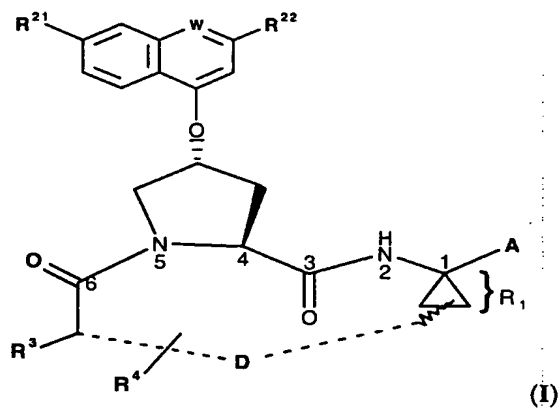
| Cpd # | R <sup>32</sup>   | R <sup>4</sup> | R <sup>22</sup>   | MS    | cell.<br>act. |
|-------|---|----------------|---|-------|---------------|
| 908   |    | H              |    | 735.2 | B             |
| 909   |    | H              |    | 777.0 | B             |
| 910   |    | H              |    | 763.1 | B             |
| 911   |    | H              |    | 777.0 | B             |
| 912   |    | H              |    | 748.9 | B             |
| 913   |   | H              |   | 762.9 | B             |
| 914   |  | H              |  | 749.0 | B             |
| 915   |  | H              |  | 751.1 | B             |
| 916   |  | 10 (R)<br>Me   | OEt   | 695.2 | B             |



## CLAIMS

What is claimed is:

1. A compound of formula (I):



- 5 wherein  $W$  is  $CH$  or  $N$ ,  
 $R^{21}$  is  $H$ , halo,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkoxy,  $C_{3-6}$  cycloalkoxy, hydroxy, or  $N(R^{23})_2$ ,  
 wherein each  $R^{23}$  is independently  $H$ ,  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;  
 $R^{22}$  is  $H$ , halo,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  thioalkyl,  $C_{1-6}$  alkoxy,  $C_{3-6}$  cycloalkoxy,  $C_{2-7}$  alkoxyalkyl,  $C_{3-6}$  cycloalkyl,  $C_{6 \text{ or } 10}$  aryl or Het, wherein Het is a  
 10 five-, six-, or seven-membered saturated or unsaturated heterocycle containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur;  
 said cycloalkyl, aryl or Het being substituted with  $R^{24}$ ,  
 wherein  $R^{24}$  is  $H$ , halo,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{1-6}$  alkoxy,  $C_{3-6}$  cycloalkoxy,  $NO_2$ ,  
 15  $N(R^{25})_2$ ,  $NH-C(O)-R^{25}$ ; or  $NH-C(O)-NH-R^{25}$ , wherein each  $R^{25}$  is independently:  $H$ ,  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;  
 or  $R^{24}$  is  $NH-C(O)-OR^{26}$  wherein  $R^{26}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;  
 $R^3$  is hydroxy,  $NH_2$ , or a group of formula  $-NH-R^{31}$ , wherein  $R^{31}$  is  $C_{6 \text{ or } 10}$  aryl, heteroaryl,  $-C(O)-R^{32}$ ,  $-C(O)-NHR^{32}$  or  $-C(O)-OR^{32}$ ,

wherein  $R^{32}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl;

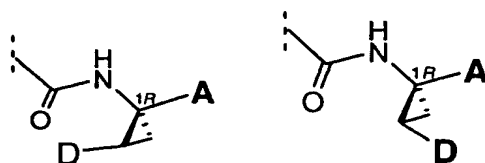
D is a 5 to 10-atom saturated or unsaturated alkylene chain optionally containing one to three heteroatoms independently selected from: O, S, or N- $R^{41}$ , wherein  $R^{41}$  is H,  $C_{1-6}$  alkyl,  $C_{3-6}$  cycloalkyl or  $-C(O)-R^{42}$ , wherein  $R^{42}$  is  $C_{1-6}$  alkyl,  $C_{3-6}$

5 cycloalkyl or  $C_{6 \text{ or } 10}$  aryl;

$R^4$  is H or from one to three substituents at any carbon atom of said chain D, said substituent independently selected from the group consisting of:  $C_{1-6}$  alkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkoxy, hydroxy, halo, amino, oxo, thio or  $C_{1-6}$  thioalkyl, and

10 A is an amide of formula  $-C(O)-NH-R^5$ , wherein  $R^5$  is selected from the group consisting of:  $C_{1-8}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{6 \text{ or } 10}$  aryl or  $C_{7-16}$  aralkyl; or A is a carboxylic acid or a pharmaceutically acceptable salt or ester thereof.

2. A compound of formula I according to claim 1, wherein said  $R^1$  moiety is  
15 selected from the 2 different diastereoisomers represented by structures (i) and (ii):

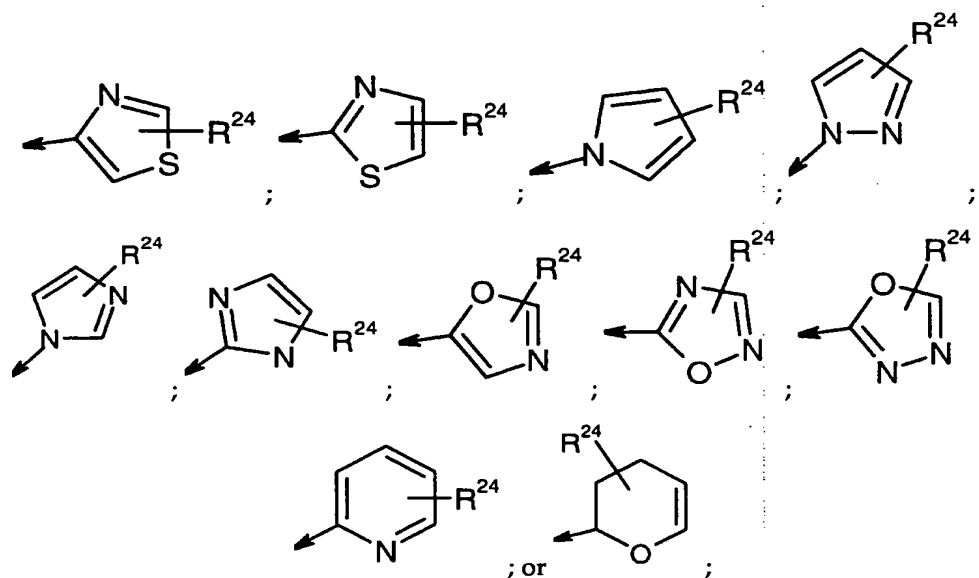


D syn to the amide (i), or D syn to A (ii).

3. The compound of formula I according to claim 2, wherein D is linked *syn* to  
20 A as represented by formula (ii).

4. The compound of formula I according to claim 1, wherein W is N;  
 $R^{21}$  is H,  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, hydroxy, chloro, or  $N(R^{23})_2$ , wherein  $R^{23}$  is H or  $C_{1-6}$  alkyl;

25  $R^{22}$  is H,  $C_{1-6}$  thioalkyl,  $C_{1-6}$  alkoxy, phenyl or Het selected from the group consisting of:



wherein  $R^{24}$  is H,  $C_{1-6}$  alkyl,  $NH-R^{25}$ ,  $NH-C(O)-R^{25}$ ,  $NH-C(O)-NH-R^{25}$ ,

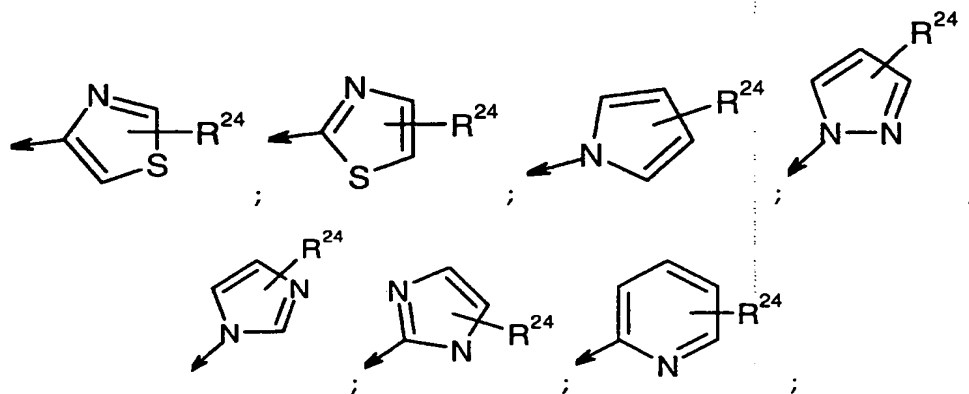
5 wherein each  $R^{25}$  is independently: H,  $C_{1-6}$  alkyl, or  $C_{3-6}$  cycloalkyl;

or  $NH-C(O)-OR^{26}$ , wherein  $R^{26}$  is  $C_{1-6}$  alkyl.

5. A compound of formula I according to claim 4, wherein  $R^{21}$  is H or  $C_{1-6}$  alkoxy.

10

6. The compound of formula I according to claim 4, wherein  $R^{22}$  is  $C_{1-4}$  alkoxy, phenyl or Het selected from the group consisting of:

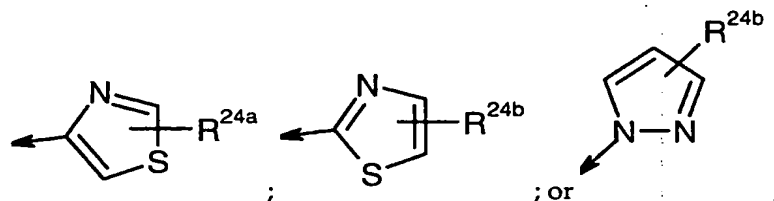


wherein  $R^{24}$  is H,  $C_{1-6}$  alkyl,  $NH-R^{25}$ , or  $NH-C(O)-R^{25}$ ;

wherein each  $R^{25}$  is  $C_{1-6}$  alkyl or  $C_{3-6}$  cycloalkyl,  
or  $NH-C(O)-OR^{26}$ , wherein  $R^{26}$  is as defined in claim 4.

5 7. A compound of formula I according to claim 6, wherein  $R^{21}$  is methoxy.

8. The compound of formula I according to claim 7, wherein  $R^{22}$  is ethoxy, or  
Het selected from the group consisting of:



10 wherein  $R^{24a}$  is  $NH-R^{25}$  or  $NH-C(O)-R^{25}$ , wherein  $R^{25}$  is  $C_{1-6}$  alkyl;  
or  $R^{24a}$  is  $NH-C(O)-OR^{26}$ , wherein  $R^{26}$  is  $C_{1-6}$  alkyl, and  $R^{24b}$  is H or  $C_{1-6}$  alkyl.

9. The compound of formula I according to claim 1, wherein  $R^3$  is an amide of  
formula  $NH-C(O)R^{32}$  or a urea of formula  $NH-C(O)-NH-R^{32}$  or a carbamate of  
15 formula  $NH-C(O)-OR^{32}$ , wherein  $R^{32}$  is  $C_{1-6}$  alkyl, or  $C_{3-6}$  cycloalkyl.

10. The compound of formula I according to claim 9, wherein  $R^3$  is a urea or a  
carbamate, wherein  $R^{32}$  is  $C_{1-6}$  alkyl or  $C_{4-6}$  cycloalkyl.

20 11. The compound of formula I according to claim 10, wherein  $R^3$  is a carbamate  
and  $R^{32}$  is *tert*-butyl, cyclobutyl or cyclopentyl.

12. The compound of formula I according to claim 1, wherein D is a 6 to 8 atom  
saturated or unsaturated alkylene chain optionally containing one or two  
25 heteroatoms independently selected from: O, S or  $N-R^{41}$ , wherein  $R^{41}$  is H,  $C_{1-6}$  alkyl,

or C<sub>2-7</sub> acyl.

13. The compound of formula I according to claim 12, wherein D optionally contains one heteroatom selected from: NH, or N-C<sub>2-7</sub> acyl.

5

14. The compound according to claim 13, wherein said heteroatom is selected from: NH or N(Ac).

10

15. The compound according to claim 13, wherein said D chain contains 7 atoms.

16. The compound according to claim 15, wherein said heteroatom is at position 10 of said D chain.

15

17. The compound according to claim 13, wherein said D chain is saturated.

18. The compound of formula I according to claim 12, wherein D is a 6 to 8 atom saturated or unsaturated alkylene chain optionally containing one heteroatom selected from: O, or S.

20

19. The compound according to claim 18, wherein said D chain contains 7 atoms.

20. The compound according to claim 19, wherein said heteroatom is at position 9 of said D chain.

25

21. The compound according to claim 20, wherein said D chain is substituted at position 8 with R<sup>4</sup>, wherein R<sup>4</sup> is H or C<sub>1-6</sub> alkyl.

22. The compound according to claim 21, wherein said R<sup>4</sup> is H or methyl.

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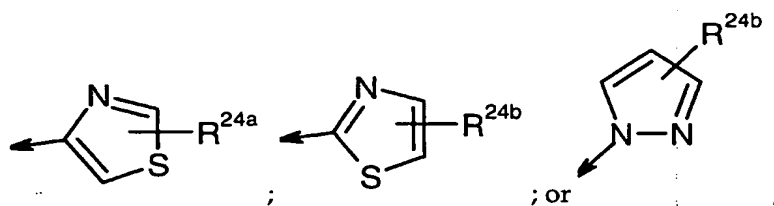
23. The compound according to claim 22, wherein said R<sup>4</sup> is H or 8-(S)-Me.

24. The compound according to claim 23, wherein said D chain is saturated.
25. The compound according to claim 19, wherein said D chain contains one double bond at position 11,12.
- 5 26. The compound according to claim 25, wherein said double bond is *trans*.
27. The compound of formula I according to claim 12, wherein D is a 6 to 8 atom, saturated or unsaturated, all carbon alkylene chain.
- 10 28. The compound of formula I according to claim 27, wherein D is a 7 atom chain.
29. The compound of formula I according to claim 28, wherein D is saturated.
- 15 30. The compound according to claim 29, wherein said D chain is substituted with R<sup>4</sup>, wherein R<sup>4</sup> is H, oxo, hydroxy, alkoxy or alkyl.
31. The compound according to claim 30, wherein said R<sup>4</sup> is H or C<sub>1-6</sub> alkyl.
- 20 32. The compound according to claim 31, wherein said R<sup>4</sup> is H or methyl.
33. The compound according to claim 32, wherein said R<sup>4</sup> is H or 10-(S)-Me.
- 25 34. The compound of formula I according to claim 28, wherein D contains one double bond.
35. The compound of formula I according to claim 34, wherein said double bond is at position 13,14 of said D chain.
- 30 36. The compound of formula I according to claim 35, wherein said double bond

is *cis*.

37. The compound according to claim 36, wherein said **D** chain is substituted with  $R^4$ , wherein  $R^4$  is H, oxo, hydroxy,  $C_{1-6}$  alkoxy or  $C_{1-6}$  alkyl.
- 5 38. The compound according to claim 37, wherein said  $R^4$  is H or  $C_{1-6}$  alkyl.
39. The compound according to claim 38, wherein said  $R^4$  is H or methyl.
- 10 40. The compound according to claim 39, wherein said  $R^4$  is H or 10-(S)-Me.
41. The compound of formula I according to claim 1, wherein **A** is a carboxylic acid.
- 15 42. A compound according to claim 1, wherein **W** is N;  
 $R^3$  is a group of formula or  $-NH-C(O)-NHR^{32}$  or  $-NH-C(O)-OR^{32}$ ,  
wherein  $R^{32}$  is  $C_{1-4}$  alkyl or  $C_{4-6}$  cycloalkyl;  
**D** is a 6 to 8 atom saturated or unsaturated alkylene chain linked to  $R^1$  *syn* to **A**,  
optionally containing one or two heteroatoms independently selected from: O, S or  
20  $N-R^{41}$ , wherein  $R^{41}$  is H, or  $C_{2-7}$  acyl;  
 $R^4$  is H, or from one to three substituents independently selected from hydroxy, or  
 $C_{1-6}$  alkyl; and  
**A** is a carboxylic acid, or a pharmaceutically acceptable salt or ester thereof.
- 25 43. The compound of formula I according to claim 42, wherein  $R^{21}$  is H or methoxy;  
 $R^{22}$  is  $C_{1-6}$  alkoxy, or Het selected from the group consisting of:

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wherein R<sup>24a</sup> is H, C<sub>1-6</sub> alkyl, NH-R<sup>25</sup>, NH-C(O)-R<sup>25</sup>; or NH-C(O)-NH-R<sup>25</sup>,

wherein R<sup>25</sup> is: H, C<sub>1-6</sub> alkyl or C<sub>3-6</sub> cycloalkyl;

or R<sup>24a</sup> is NH-C(O)-OR<sup>26</sup>, wherein R<sup>26</sup> is C<sub>1-6</sub> alkyl or C<sub>3-6</sub> cycloalkyl;

5 and R<sup>24b</sup> is H or C<sub>1-6</sub> alkyl;

R<sup>3</sup> is urea of the formula N-C(O)-NHR<sup>32</sup> or a carbamate of the formula N-C(O)-OR<sup>32</sup>,

wherein R<sup>32</sup> is C<sub>1-6</sub> alkyl or C<sub>3-6</sub> cycloalkyl;

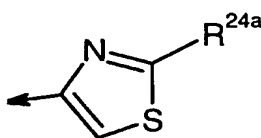
D is a 7-atom alkylene chain optionally containing one double bond at position 11,12 or 13,14;

10 said D chain optionally containing one heteroatom independently selected from: O, S, NH, N(Me), or N(Ac); and

R<sup>4</sup> is H or C<sub>1-6</sub> alkyl.

44. The compound of formula I according to claim 43, wherein R<sup>21</sup> is methoxy,

15 and R<sup>22</sup> is ethoxy or



wherein R<sup>24a</sup> is NH-(C<sub>1-4</sub> alkyl); NH-C(O)-(C<sub>1-4</sub> alkyl); NH-C(O)-O-(C<sub>1-4</sub> alkyl); or

NH-C(O)-NH-(C<sub>1-4</sub> alkyl);

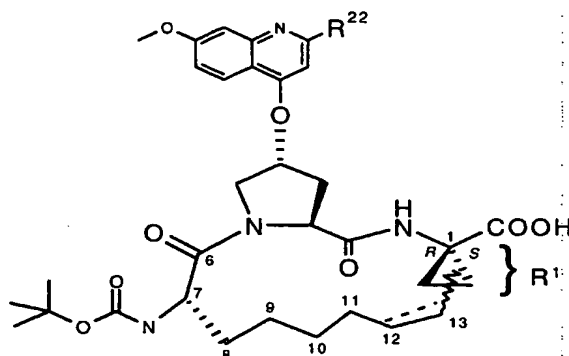
D is a C7 all carbon chain, saturated or containing one *cis* double bond at position

20 13,14.



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45. A compound of formula:

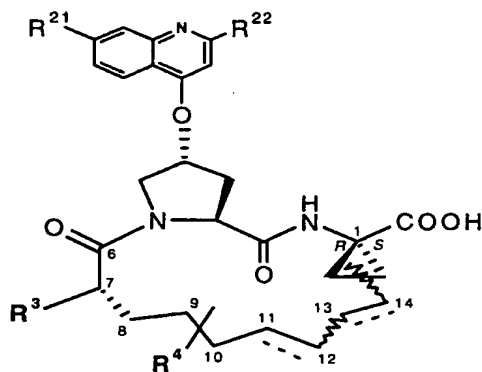


comprising a single stereoisomer at  $R^1$ , wherein said double bond, D- $R^1$  bond stereochemistry and  $R^{22}$  are defined as follows:

| Cpd. # | double bond:        | D- $R^1$ bond stereochem:          | $R^{22}$ : |
|--------|---------------------|------------------------------------|------------|
| 101    | 12,13- <i>trans</i> | 1 <i>R</i> , D <i>syn</i> to amide | phenyl;    |
| 102    | none                | 1 <i>R</i> , D <i>syn</i> to acid  | phenyl;    |
| and    |                     |                                    |            |
| 103    | none                | 1 <i>R</i> D <i>syn</i> to amide   | phenyl.    |

5

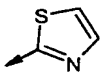
46. A compound of formula:



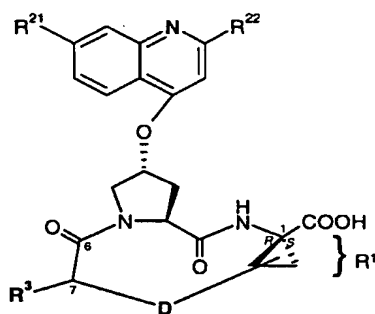
comprising a single stereoisomer at  $R^1$ , wherein  $R^3$ ,  $R^4$ , said double bond position, D- $R^1$  bond stereochemistry,  $R^{21}$  and  $R^{22}$  are defined as follows:

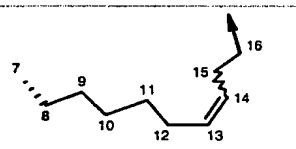
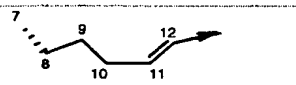
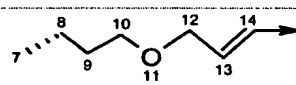
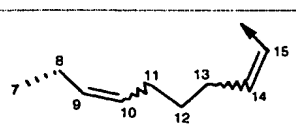
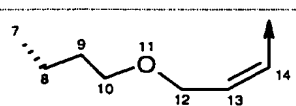
| Cpd # | R <sup>3</sup> :            | R <sup>4</sup> :             | double bond:           | D-R <sup>1</sup> bond stereochemistry: | R <sup>21</sup> : | R <sup>22</sup> : |
|-------|-----------------------------|------------------------------|------------------------|--|-------------------|-------------------|
| 202   | NH-Boc                      | H                            | 11,12-<br><i>trans</i> | 1R or 1S,<br>D syn to acid             | H                 | H;                |
| 203   | NH-acetyl                   | H                            | 11,12-<br><i>trans</i> | 1R or 1S,<br>D syn to acid             | H                 | H;                |
| 205   | NH-Boc                      | 11-OH<br>12-OH<br><i>cis</i> | none                   | 1R or 1S,<br>D syn to acid             | H                 | H;                |
| 206   | NH-Boc                      | H                            | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | H                 | H;                |
| 207   | NH-Boc                      | H                            | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | H;                |
| 208   | NH-Boc                      | H                            | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | phenyl;           |
| 209   | NH-C(O)-<br>NH- <i>t</i> Bu | H                            | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | phenyl;           |
| 210   | NH-Boc                      | H                            | 13,14-<br><i>cis</i>   | 1S, D syn to acid                      | OMe               | phenyl;           |
| 211   | NH <sub>2</sub>             | H                            | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | phenyl;           |
| 213   | OH (one isomer)             | H                            | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | H;                |
| 214   | NH-Boc                      | 10-oxo                       | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | phenyl;           |
| 215   | NH-Boc                      | H                            | none                   | 1R, D syn to acid                      | OMe               | phenyl;           |
| 217   | NH-Boc                      | 10-OH<br>(mixt dia stereo)   | 13,14-<br><i>cis</i>   | 1R, D syn to acid                      | OMe               | phenyl;           |
| 218   | NH-Boc                      | 10-oxo                       | 13,14-<br><i>cis</i>   | 1R, D syn to amide                     | OMe               | phenyl;           |
| 219   | NH-Ac                       | H                            | none                   | 1R, D syn to acid                      | OMe               | phenyl;           |

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| Cpd #   | R <sup>3</sup> : | R <sup>4</sup> : | double bond: | D-R <sup>1</sup> bond stereochemistry: | R <sup>21</sup> : | R <sup>22</sup> :   |
|---------|------------------|------------------|--------------|--|-------------------|---|
| and 220 | NH-Boc           | H                | 13,14-cis    | 1R, D syn to amide                     | OMe               |  |

47. A compound of formula:

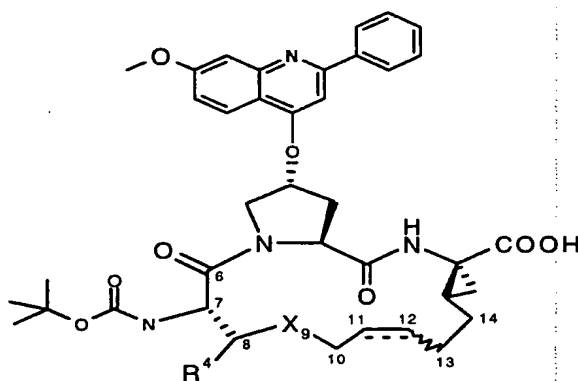
comprising a single stereoisomer at R<sup>1</sup>, wherein R<sup>3</sup>, D, D-R<sup>1</sup> bond stereochemistry,5 R<sup>21</sup> and R<sup>22</sup> are defined as follows:

| Cpd. # | R <sup>3</sup> : | - D -:  | D-R <sup>1</sup> bond stereochemistry: | R <sup>21</sup> : | R <sup>22</sup> : |
|--------|------------------|---|--|-------------------|-------------------|
| 301    | NH-Boc           |  | 1R or 1S,<br>D syn to acid             | H                 | H;                |
| 302    | NH-Boc           |  | 1R, D syn to amide                     | OMe               | Ph;               |
| 303    | NH-Boc           |  | 1R, D syn to amide                     | OMe               | Ph;               |
| 304    | NH-Boc           |  | 1R, D syn to acid                      | OMe               | Ph;               |
| 305    | HO               |  | 1R, D syn to acid                      | OMe               | Ph;               |

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| Cpd. #  | R <sup>3</sup> : | - D -: | D-R <sup>1</sup> bond stereochemistry: | R <sup>21</sup> : | R <sup>22</sup> : |
|---------|------------------|--------|--|-------------------|-------------------|
| 306     | NH-Boc           |        | 1R, D <i>syn</i> to amide              | OMe               | Ph;               |
| 307     | NH-Boc           |        | 1R, D <i>syn</i> to acid               | OMe               |                   |
| and 308 | NH-Ac            |        | 1R, D <i>syn</i> to acid               | OMe               | OEt.              |







48. A compound of formula:



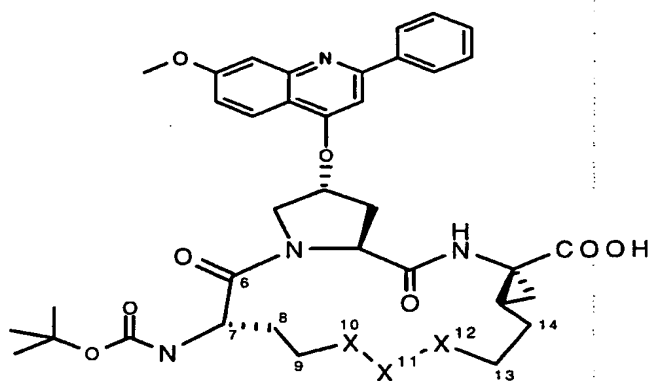
wherein said D-R<sup>1</sup> bond is *syn* to acid, R<sup>4</sup>, X<sub>9</sub>, and said 11,12 double bond are defined as follows:

| Cpd # | R <sup>4</sup> : | X <sub>9</sub> : | 11, 12 double bond: |
|-------|------------------|------------------|---------------------|
| 401   | H                | CH <sub>2</sub>  | trans;              |
| 402   | H                | CH <sub>2</sub>  | cis;                |
| 403   | H                | O                | trans;              |
| 404   |                  | O                | trans;              |

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| Cpd #   | R <sup>4</sup> :  | X <sub>9</sub> : | 11, 12 double bond: |
|---------|---|------------------|---------------------|
| 405     |    | O                | trans;              |
| 406     | H   | O                | none;               |
| 407     |    | O                | none;               |
| 408     |    | O                | none;               |
| 409     |    | O                | cis;                |
| 410     |    | S                | trans;              |
| 411     |  | S                | cis;                |
| and 412 | 8-(Me) <sub>2</sub>   | 9-S              | cis.                |

49. A compound of formula:

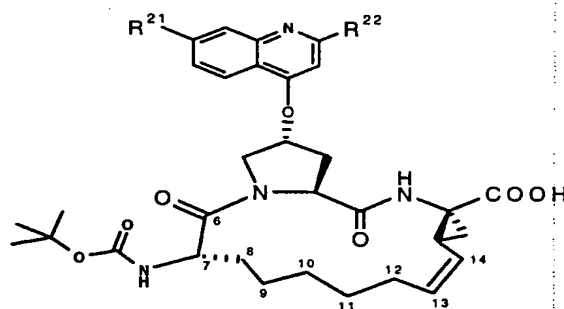


wherein said D-R<sup>1</sup> bond is *syn* to acid, X<sub>10</sub>, X<sub>11</sub>, and X<sub>12</sub> are defined as follows:

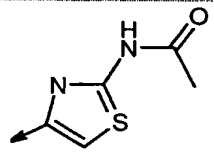
140

| Cpd #      | X <sub>10</sub> : | X <sub>11</sub> : | X <sub>12</sub> : |
|------------|-------------------|-------------------|-------------------|
| 501        | CH <sub>2</sub>   | O                 | CH <sub>2</sub> ; |
| 502        | CH <sub>2</sub>   | CH <sub>2</sub>   | CH <sub>2</sub> ; |
| 503        | CH <sub>2</sub>   | CH <sub>2</sub>   | NH;               |
| 504        | CH <sub>2</sub>   | CH <sub>2</sub>   | N(Me);            |
| 505        | CH <sub>2</sub>   | CH <sub>2</sub>   | N(CO)Me;          |
| 506        | CH <sub>2</sub>   | CH <sub>2</sub>   | N(CO)Ph;          |
| 507        | NH                | CH <sub>2</sub>   | CH <sub>2</sub> ; |
| and<br>508 | N(CO)Me           | CH <sub>2</sub>   | CH <sub>2</sub> . |

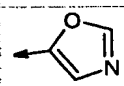
50. A compound of formula:



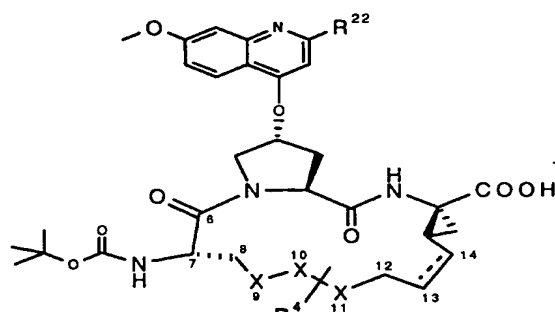
wherein said D-R<sup>1</sup> bond is *syn* to acid, R<sup>21</sup> and R<sup>22</sup> are defined as follows:

| Cpd # | R <sup>21</sup> :  | R <sup>22</sup> :  |
|-------|--------------------|--|
| 601   | N(Me) <sub>2</sub> |  ; |
| 602   | OH                 | (CF <sub>3</sub> );  |

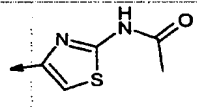
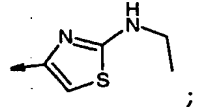
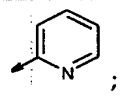
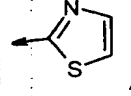
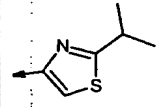
141

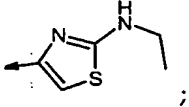
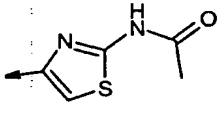
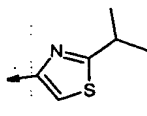
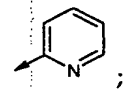
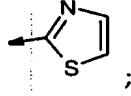

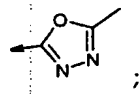
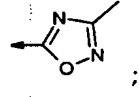
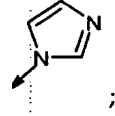
| Cpd #      | R <sup>21</sup> : | R <sup>22</sup> :   |
|------------|-------------------|---|
| and<br>603 | OMe               |  |

51. A compound of formula:

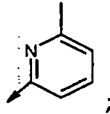
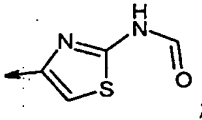
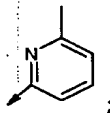
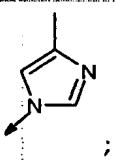
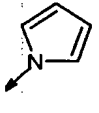
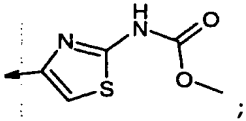
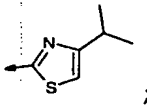
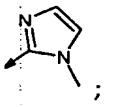


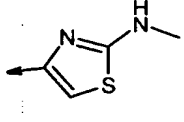
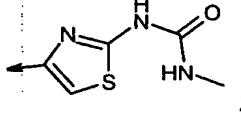
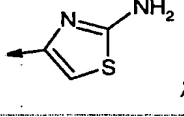
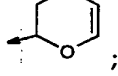
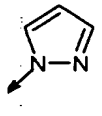
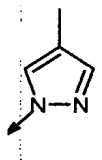
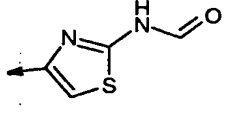
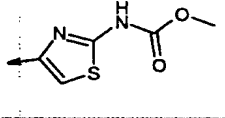
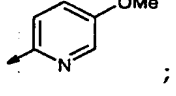
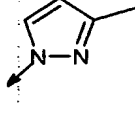
wherein said D-R<sup>1</sup> bond is *syn* to acid, R<sup>4</sup>, X<sub>9</sub>, X<sub>10</sub>, X<sub>11</sub>, said 13,14 double bond and R<sup>22</sup> are defined as follows:

| Cpd # | R <sup>4</sup> : | X <sub>9</sub> ; X <sub>10</sub> or<br>X <sub>11</sub> : | 13,14 double bond: | R <sup>22</sup> :   |
|-------|------------------|--|--------------------|---|
| 701   | H                | 11-O   | Cis                | phenyl;   |
| 702   | H                | CH <sub>2</sub>  | Cis                |  ; |
| 703   | H                | CH <sub>2</sub>  | None               |  ; |
| 704   | H                | CH <sub>2</sub>  | Cis                |  ; |
| 705   | H                | CH <sub>2</sub>  | Cis                |  ; |
| 707   | H                | CH <sub>2</sub>  | Cis                |  ; |

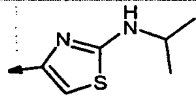
| Cpd # | R <sup>4</sup> : | X <sub>9</sub> , X <sub>10</sub> or X <sub>11</sub> : | 13,14 double bond: | R <sup>22</sup> :   |
|-------|------------------|---|--------------------|---|
| 708   | H                | CH <sub>2</sub>                                       | Cis                |  ;   |
| 709   | H                | CH <sub>2</sub>                                       | None               |  ;   |
| 710   | H                | CH <sub>2</sub>                                       | None               |  ;   |
| 711   | H                | CH <sub>2</sub>                                       | None               |  ;   |
| 712   | H                | CH <sub>2</sub>                                       | Cis                | -OEt;   |
| 713   | H                | CH <sub>2</sub>                                       | None               |  ;  |
| 714   | H                | CH <sub>2</sub>                                       | None               | -OEt;   |
| 715   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 716   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 717   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 718   | H                | CH <sub>2</sub>                                       | Cis                |  ; |



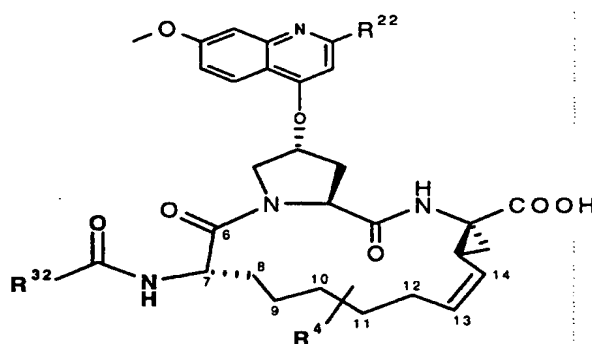
| Cpd # | R <sup>4</sup> : | X <sub>9</sub> ; X <sub>10</sub> or<br>X <sub>11</sub> : | 13,14 double bond: | R <sup>22</sup> :   |
|-------|------------------|--|--------------------|---|
| 719   | H                | CH <sub>2</sub>  | Cis                |  ;   |
| 720   | H                | CH <sub>2</sub>  | None               |  ;   |
| 721   | H                | CH <sub>2</sub>  | None               |  ;   |
| 722   | H                | CH <sub>2</sub>  | Cis                |  ;   |
| 723   | H                | CH <sub>2</sub>  | None               |  ; |
| 724   | H                | CH <sub>2</sub>  | None               |  ; |
| 725   | H                | CH <sub>2</sub>  | Cis                |  ; |
| 726   | H                | CH <sub>2</sub>  | Cis                |  ; |
| 727   | H                | CH <sub>2</sub>  | Cis                | -CH <sub>2</sub> -OMe;  |
| 728   | H                | CH <sub>2</sub>  | Cis                | Me;   |

| Cpd # | R <sup>4</sup> : | X <sub>9</sub> , X <sub>10</sub> or X <sub>11</sub> : | 13,14 double bond: | R <sup>22</sup> :   |
|-------|------------------|---|--------------------|---|
| 729   | H                | CH <sub>2</sub>                                       | Cis                |  ;   |
| 730   | H                | CH <sub>2</sub>                                       | None               |  ;   |
| 731   | H                | CH <sub>2</sub>                                       | Cis                |  ;   |
| 732   | H                | CH <sub>2</sub>                                       | Cis                |  ;   |
| 733   | H                | CH <sub>2</sub>                                       | Cis                |  ;  |
| 734   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 735   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 736   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 737   | H                | CH <sub>2</sub>                                       | Cis                |  ; |
| 738   | H                | CH <sub>2</sub>                                       | Cis                |  ; |

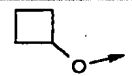
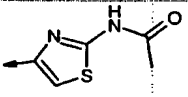
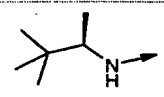
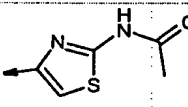
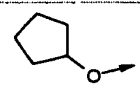
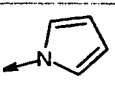
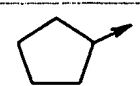
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| Cpd #   | R <sup>4</sup> : | X <sub>9</sub> , X <sub>10</sub> or X <sub>11</sub> : | 13,14 double bond: | R <sup>22</sup> :   |
|---------|------------------|---|--------------------|---|
| 739     | 10-(R) Me        | CH <sub>2</sub>                                       | none               | Ph;   |
| 740     | 10-(S) Me        | CH <sub>2</sub>                                       | none               | Ph;   |
| and 741 | H                | CH <sub>2</sub>                                       | Cis                |  |

52. A compound of formula:



wherein said D-R<sup>1</sup> bond is *syn* to acid, said 13,14 double bond is *cis*, R<sup>32</sup>, R<sup>4</sup> and R<sup>22</sup> are defined as follows:

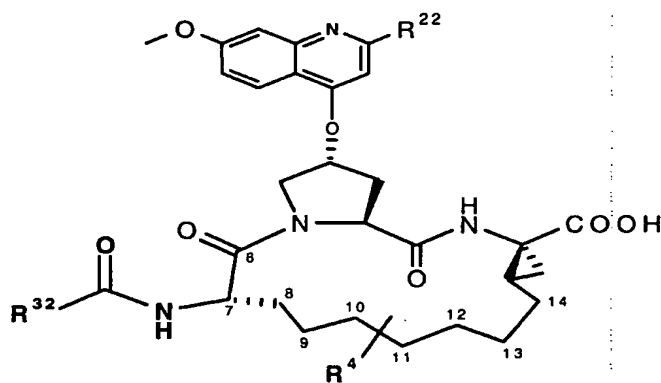
| Cpd # | R <sup>32</sup> :   | R <sup>4</sup> : | R <sup>22</sup> :  |
|-------|---|------------------|--|
| 801   |  | H                |  ; |
| 803   | <i>n</i> -Pr  | H                | OE <sub>t</sub> ;  |
| 804   |  | H                |  ; |
| 805   |  | H                |  ; |
| 806   |  | H                | OE <sub>t</sub> ;  |

| Cpd # | R <sup>32</sup> : | R <sup>4</sup> : | R <sup>22</sup> : |
|-------|-------------------|------------------|-------------------|
| 807   |                   | H                | OEt;              |
| 808   |                   | H                | OEt;              |
| 809   |                   | H                | ;                 |
| 810   |                   | H                | ;                 |
| 811   |                   | H                | ;                 |
| 812   |                   | H                | ;                 |
| 813   |                   | H                | OEt;              |
| 814   |                   | H                | ;                 |
| 815   |                   | H                | ;                 |
| 816   |                   | H                | ;                 |
| 817   |                   | H                | ;                 |
| 818   |                   | H                | ;                 |

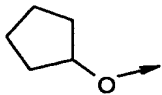
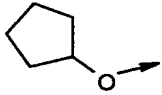
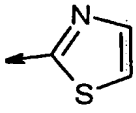
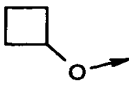
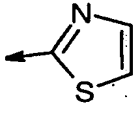
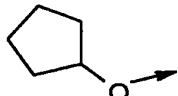
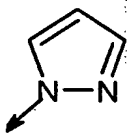
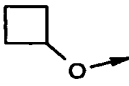
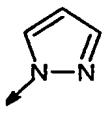
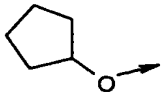
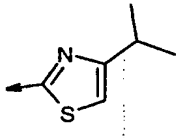
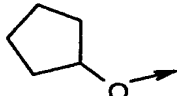

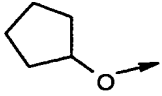
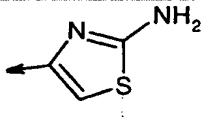
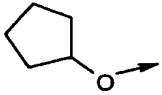
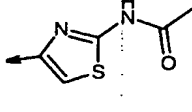
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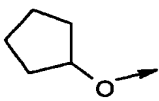
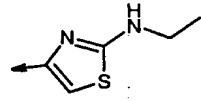
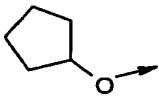
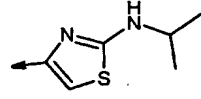
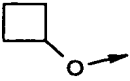
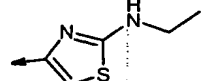

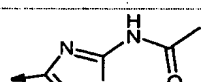
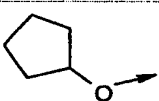
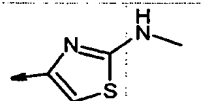
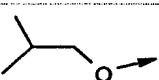
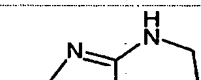
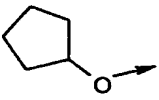
| Cpd #      | R <sup>32</sup> : | R <sup>4</sup> : | R <sup>22</sup> : |
|------------|-------------------|------------------|-------------------|
| 819        |                   | H                |                   |
| 820        |                   | H                |                   |
| 821        |                   | H                |                   |
| 822        |                   | H                |                   |
| 823        |                   | H                |                   |
| and<br>824 |                   | 10- (R) Me       | OEt.              |

53. A compound of formula:



wherein said D-R<sup>1</sup> bond *syn* to acid, R<sup>32</sup>, R<sup>4</sup> and R<sup>22</sup> are defined as follows:

| Cpd # | R <sup>32</sup> :   | R <sup>4</sup> : | R <sup>22</sup> :  |
|-------|---|------------------|--|
| 901   |    | H                | OEt;   |
| 902   |    | H                |  ;   |
| 903   |    | H                |  ;   |
| 904   |    | H                |  ;   |
| 905   |   | H                |  ;  |
| 906   |  | H                |  ; |
| 907   |  | H                |  ; |
| 908   |  | H                |  ; |
| 909   |  | H                |  ; |

| Cpd #      | R <sup>32</sup> :   | R <sup>4</sup> : | R <sup>22</sup> :  |
|------------|---|------------------|--|
| 910        |    | H                |  ;   |
| 911        |    | H                |  ;   |
| 912        |    | H                |  ;   |
| 913        |    | H                |  ;   |
| 914        |    | H                |  ;   |
| 915        |  | H                |  ; |
| and<br>916 |  | 10 (R) Me        | OEt.   |

54. A method of inhibiting the replication of hepatitis C virus by exposing the virus to a hepatitis C viral NS3 protease inhibiting amount of the compound of formula I according to claim 1.

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55. A pharmaceutical composition comprising an anti-hepatitis C virally effective amount of a compound of formula I according to claim 1, or a therapeutically acceptable salt or ester thereof, in admixture with a pharmaceutically acceptable carrier medium or auxiliary agent.

10

56. The pharmaceutical composition according to claim 55, further comprising

an additional immunomodulatory agent.

57. The pharmaceutical composition according to claim 56, wherein said additional immunomodulatory agents is selected from the group consisting of:  $\alpha$ -,  $\beta$ -  
5 , and  $\delta$ -interferons.

58. The pharmaceutical composition according to claim 55, further comprising an antiviral agent.

10 59. The pharmaceutical composition according to claim 58, wherein said antiviral agent is selected from the group consisting of: ribavirin and amantadine.

60. The pharmaceutical composition according to claim 55, further comprising another inhibitor of HCV protease.  
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61. The pharmaceutical composition according to claim 55, further comprising an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase, or metalloprotease.

20 62. Use of pharmaceutical composition according to claim 55, for the manufacture of a medicament for treating a hepatitis C viral infection in a mammal.



# INTERNATIONAL SEARCH REPORT

Int .tional Application No  
PCT/CA 00/00353

|  |   |   |   |   |
|--|---|---|---|---|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC 7 C07K5/08 C07K5/078 A61K38/05 A61K38/06 A61P31/14   |   |   |   |   |
| According to International Patent Classification (IPC) or to both national classification and IPC  |   |   |   |   |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC 7 C07K A61K  |   |   |   |   |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  |   |   |   |   |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)   |   |   |   |   |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>  |   |   |   |   |
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.   |   |   |
| A  | WO 99 07733 A (BOEHRINGER INGELHEIM CA LTD<br>;RANCOURT JEAN (CA); SIMONEAU BRUNO (C)<br>18 February 1999 (1999-02-18)<br>cited in the application<br>the whole document<br>-----   | 1-62  |   |   |
| <input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.   |   |   |   |   |
| * Special categories of cited documents :  |   |   |   |   |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;">           "A" document defining the general state of the art which is not considered to be of particular relevance<br/>           "E" earlier document but published on or after the international filing date<br/>           "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br/>           "O" document referring to an oral disclosure, use, exhibition or other means<br/>           "P" document published prior to the international filing date but later than the priority date claimed         </td> <td style="width: 50%; vertical-align: top;">           "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br/>           "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br/>           "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br/>           "&amp;" document member of the same patent family         </td> </tr> </table> |   |   | "A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier document but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br>"&" document member of the same patent family |
| "A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier document but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed  | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.<br>"&" document member of the same patent family |   |   |   |
| Date of the actual completion of the international search<br><br><div style="text-align: center;">16 June 2000</div>   |   | Date of mailing of the international search report<br><br><div style="text-align: center;">26/06/2000</div> |   |   |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, T.x. 31 651 epo nl.<br>Fax: (+31-70) 340-3016  |   | Authorized officer<br><br><div style="text-align: center;">Groenendijk, M.</div>                            |   |   |

# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/CA 00/00353

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)   | Publication<br>date      |
|---|---------------------|------------------------------|--------------------------|
| WO 9907733 A                              | 18-02-1999          | AU 8795698 A<br>EP 1003775 A | 01-03-1999<br>31-05-2000 |